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ON THE EVOLUTION OF THE AUDITORY CONDUCTING APPARATUS: A NEW THEORY BASED ON FUNCTIONAL CONSIDERATIONS

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INTRODUCTION

The problem of the evolution of the middle ear is one of abiding interest. Authorities still differ on many points but there is general agreement on the basic facts. The sauropsid columella or stapes is derived from the piscine hyomandibular whilst the mammalian incus and malleus represent the sauropsid quadrate and articular respectively. These bare anatomical facts are sufficiently bizarre to prompt the question, "Why did they occur?" This —the physiological or functional point of view—has received far too little attention.

The Classical or Anatomical Theory

Many authorities content themselves with locating the stapes in the particular fossil skull under consideration. Then a "middle ear cavity" is arbitrarily envisaged surrounding the bone—and finally a tympanic membrane is envisaged wherever the "middle ear cavity" reaches the surface (fig. 1). Unfortunately, this purely anatomical approach, so fruitful in other fields of palaeontology, has led to grievous error because of its disregard of physiology. Its basic assumption that the middle ear is necessarily an air-sensitive mechanism is false. It is true that the middle ear in most important modern animals is of that type. That is to say, it comprises a tympanic membrane which can pick up air-borne sound and an ossicular chain—freely suspended in a middle ear cavity—to convey the drum vibrations to the inner ear. Such a mechanism, however, is by no means universal. Many less conspicuous groups have hearing mechanisms which comprise neither drum nor middle ear cavity—a familiar example is the snake.

Air Conduction and Bone Conduction

Such animals hear by bone conduction. The reader, by placing the stem of a tuning fork on his forehead, can confirm that bone conduction is also present in the mammal—as indeed it is in all animals that can hear at all. For the detection of air-borne sound the drum mechanism is more efficient than the bone mechanism but in the detection of substrate vibrations, the bone mechanism is superior—a fact quite familiar to the Red Indian when he puts his ear to the ground. It is known that the earliest amphibia—e.g. *Eogyrinus* —were completely prostrate and the march

of evolution has seen, first the elevation of the head, then the shoulders and then—by various means—the emergence of the quadrupedal and bipedal postures. This—in the writer's opinion—is one of the many "factors" or "biological forces" that have profoundly influenced the evolution of the middle ear. It leads to the broad conclusion that the early amphibia heard by bone conduction and that air conduction was a later development necessitated by the assumption of an erect posture which by elevating the head rendered the bone mechanism useless. Posture, however, was only one of the many contributory factors, and in the present article I shall refer to the following:

1. Modification in the central nervous system.
2. Changes in mastication and deglutition.
3. Absolute size.

It is to be hoped that further study will throw light on other factors—but whatever happens the coordinating conception must surely be that of "function."

Function—Orthogenesis—or Macro-evolution

In the case of the middle ear, the functional approach has possibly been neglected because of difficulties in its application. It is not easy to envisage the metamorphosis of the hyomandibular into the stapes, or of the articulo-quadrate complex into the auditory ossicles. This situation is familiar to all students of evolution. A primeval organ A is believed, on good grounds, to have given rise to a modern organ B. A and B have well known and quite different functions, but it is difficult, if not impossible, to envisage how the transition can have taken place since—in the transition stages—the structures would be neither one thing nor the other. It is no solution to this problem to ignore it as in the anatomical method quoted above. Nor does the word "orthogenesis" help us—implying as it does an unknown process determining the course of evolution for no apparent reason. "Macro-evolution" is another word which in effect begs the question. Watson (1953) quite correctly rejects the idea of macro-evolution.

> "The change of function from jaw articulation to hearing is exactly the kind of matter which has been held to demand a special 'macro-evolution' mechanism, distinct from and of a different nature to 'natural selection.' Those who believe in the existence of such macro-evolution, appear to hold that it is brought about suddenly perhaps by a single change in the genetic mechanism, that at a single jump calls into being a new major group, or a structure or organ for which it is difficult to conceive the intermediary stages by which it could have been derived from an earlier structure.
>
> "The sound-conducting mechanism of the mamalian ear is such a structure. The struggles of embryologists to invent a method of conversion of a lizard skull, with its movable quadrate, into that of a mammal, show that it is not easy to conceive the transformation without knowledge of the actual intermediate conditions. Indeed the mammalian ear is a typical event of the kind attributed to macro-evolution. But the story set out above shows that the change is brought about, without any reference to hearing for the greater part of the process, to improve the jaw apparatus and to get rid of a skull inconveniently deep. There is no reason at all to postulate any unusual mechanism for the evolution of the mammalian sound-transmitting apparatus. The changes which lead to its formation occur over a period of many millions of years and generations owe their nature to ordinary mechanical needs having nothing to do with hearing" (ibid., p. 176).

The conception of function is implicit in this argument but has been applied in a very restricted sense and it is difficult to accept seriously the suggestion that the middle ear emerged almost as an accidental by-product of the changing *mechanical* needs of the skull. Let us not forget that hearing is one of the great exteroceptor senses enabling the ainmal to detect and *locate* its enemy, prey or mate—by night as well as by day. It is thus immensely valuable in the struggle for existence. Furthermore, hearing is almost universal. Science today, with improved techniques, is learning to recognise that many creatures previously thought to be deaf, have

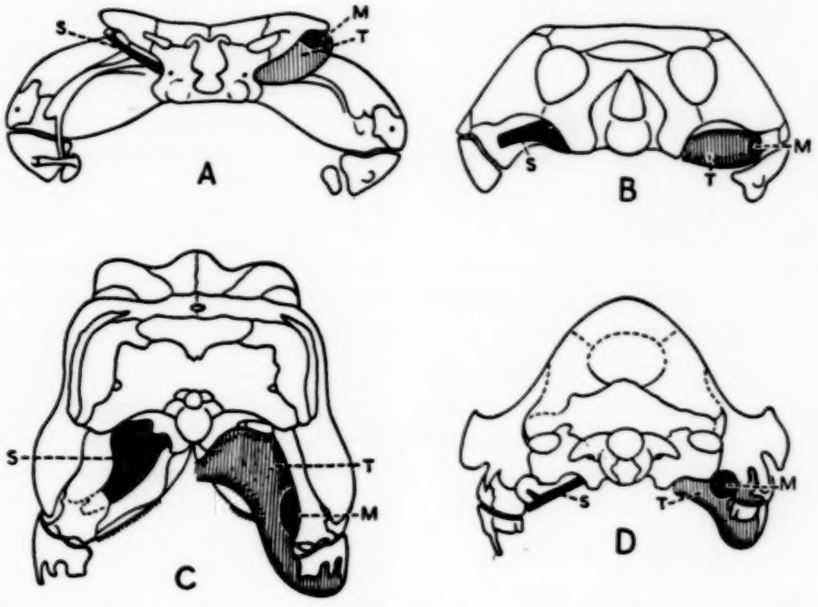

FIG. 1. The anatomical theory. A: Stegocephalian. B: Cotylosaur. C.: Pelycosaur. D: Therocephalian. In each figure the columella or stapes S is shown on the left. The hypothetical tympanic cavity T is shown on the right. Wherever it reaches the surface a tympanic membrane M is envisaged. (Adapted from Westoll, Proc. Roy. Soc. B, 1943, 131, 393.)

hearing adequate to their needs and in many cases of surpassing efficiency. For example, although Weber described the auditory ossicles in the Ostariophysi over a hundred years ago, many writers still speak of most fishes as deaf. Pumphrey (1950), however, has recently argued very convincingly that the lateral line organ is an auditory mechanism, perfectly suited to an aquatic environment. Nor is "hearing" confined to the vertebrates—many varieties of insects being responsive to sound. It is noteworthy that the insects have evolved *functionally* in a manner closely comparable with that of the vertebrates. Starting with proprioceptors (comparable with the vertebrate static labyrinth) they evolved exteroceptors sensitive to substrate vibrations. Subsequently—with the evolution of a tympanic membrane—they acquired true AC hearing. Pumphrey (1940) says:

"Chordotonal sensilla occur throughout the insects, in larvae as well as adults, though tympanic organs occur only in adults and are sporadically distributed. It seems relatively certain, although there is no direct evidence that the primitive function of chordotonal sensilla is a proprioceptive one—to register the displacement of one part of the skeleton with respect to another. The development of a special proprioceptive function as in the wing bases and halters of Diptera or an exteroceptive function as in tympanic organs and perhaps in Johnson's organ is a subsequent adaptation of a pre-existing system to meet a special need. The presence of elaborate chordotonal organs in the tibiae of Hymenoptera which have no tympanic organs and in the tibiae of the second and third legs of tettigoniids and gryllids in a position corresponding to that of the tympanic organs of the first pair is very suggestive. It may well be the case that the tibial chordotonal organs were developed as receptors for vibratory stimuli transmitted through the substrate, before association with a tympanic membrane rendered them more specifically sensitive to airborne sounds."

It is impossible to believe that a function of such universal distribution and vital importance could have fallen into abeyance for prolonged periods as is suggested by anatomical theory (see p. 221). On the contrary, as the writer's theory is unfolded, we shall see how hearing has at all times played an important, if not decisive, role in the course of evolution. Thus this present challenge to accepted theory is based primarily on functional grounds. In addition, however, there is valuable ontogenetic evidence to consider and indeed

the facts of morphology itself must be reviewed.

The Testimony of Morphology

In speculating as to how extinct animals heard, we may glean some evidence by studying how modern animals hear. In the present brief survey of modern middle ear mechanisms I shall confine myself to the terrestrial vertebrates—merely noting, as mentioned above, that the auditory function is widespread not only in insects but also in aquatic life both vertebrate and invertebrate. First, however, it is necessary to agree on a definition of middle ear and for the present purpose I define it as comprising "All structures intervening between the environment and the inner ear." This disregards the somewhat artificial anatomical distinction between middle and outer ear. The outer ear is by no means universal and its presence or absence in no way affects the argument. Similarly, the boundary between middle and inner ears is not easily defined—but for present purposes I place it at the oval window. The definition proposed leads to surprising if not bizarre conclusions. For example, in certain animals the lower jaw, the skull or the whole fore-leg act as sound conductors and so constitute part of the middle ear. No theory of the middle ear can be acceptable if it fails to account for these extraordinary mechanisms. In the account of these mechanisms I have adopted a nomenclature based on the stapes. In every case the inner end of the stapes contacts the vestibule of the inner ear at the oval window. The mechanisms merely differ in the outer attachment of the stapes. When, for example, this is at the quadrate I have called it V.Q., i.e. Vestibulo-Quadrate. The exception is V.S. in which there is no true stapes. The title Vestibulo-Scapular, however, serves well enough to distinguish this particular mechanism. A survey of modern animals reveals the following six different auditory conducting mechanisms (Fig. 2).

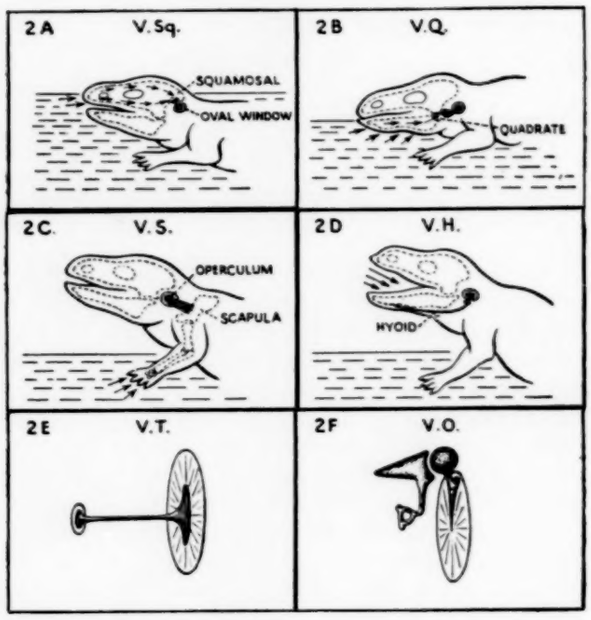

Fig. 2. Six different auditory mechanisms. 2A. Vestibulo Squamosal—a bone-conducting mechanism. The columella runs from oval window to squamosal. No tympanic cavity or membrane—hearing via dermal skull bones. Found in urodeles and early amphibia. 2B. Vestibulo Quadrate similar to V.Sq. but columella joins quadrate instead of squamosal. Hearing via lower jaw. Found in some lizards, snakes and many early reptiles. 2C. Vestibulo Scapular. Hearing via the manus—a bone conducting device. Found in modern amphibia. 2D. Vestibulo Hyoid. A dual purpose mechanism. Primarily acting as a bone conductor in a prostrate animal, it can also act as a crude detector of air-borne sound when the animal is erect. Found in Sphenodon and primitive Archosaurs. 2E. Vestibulo Tympanic. The air sensitive monossicular mechanism found in birds, frogs and many reptiles. 2F. Vestibulo Ossicular. The air sensitive triple ossicle mechanism of the mammal.

1. *V.Sq. Vestibulo-Squamosal* (fig. 2A). This is found in urodeles. The columella stretches from oval window to the squamosal. It is a bone-conducting mechanism obviously adapted to a semi-submerged or burrowing existence. The animal picks up vibrations by direct contact between the skull and substrate. There is no tympanic membrane and no middle ear cavity.

2. *V.Q. Vestibulo-Quadrate* (fig. 2B). Found in snakes—some lizards and many extinct reptiles. This resembles V.Sq., but the columella is attached externally to the quadrate. Vibrations are transmitted via the lower jaw and articulo-quadrate joint. This is obviously adapted to a life *on* rather than under the

ground. It only works when the animal is prostrate.

3. *V.S. Vestibulo-Scapular* (fig. 2C). This remarkable mechanism is only found in urodeles and anura. It consists of an operculum, i.e. a plaque of cartilage lying in the oval window posterior to the true stapes. The musculus opercularis runs from the operculum to the scapula. Vibrations in the substrate pass via the fore-paw to the scapula and thence via the opercular structures to the vestibule. This is also a bone-conducting mechanism but it does allow its owner to raise the head off the ground.

4. *V.H. Vestibulo-Hyoid* (fig. 2D). Hearing via the Hyoid bone. This resembles V.Q., but the hyoid is the intermediate conductor instead of the lower jaw. This mechanism is essentially bone-conducting but it can also act as a crude air-sensitive device in which the floor of the mouth acts as a tympanic membrane. This dual function has played a vital role in the evolution of the sauropsid middle ear.

5. *V.T. Vestibulo-Tympanic* (fig. 2E), i.e. hearing via a perfected drum and a single ossicle. In the Anura, in many reptiles and in birds the stapes (usually called the columella) is a delicate spicule of bone freely mobile in a middle ear cavity. Its inner extremity fits into the oval window—its outer extremity is continuous with a cartilaginous extra columella which is inserted into a perfected trileminate drum-skin.

6. *V.O. Vestibulo-Ossicular* (fig. 2F). This is the mammalian triple osicle system.

Transitional Mechanisms

In addition to the above distinct mechanisms it is possible to recognise several transitional types. *Sphenodon,* a primitive New Zealand lizard, represents a transition from V.H. to V.T. The stapes not only connects with the hyoid but also with a "plaque" of fibrous tissue. This "plaque" is attached circumferentially to the episthotic and squamosal, the outer plate of the quadrate, the posterior edge of the quadrato-jugal, the retro-articular process of the lower jaw as well as the hyoid itself. It lies deep to the skin and subcutaneous tissues and for the most part is covered also by the M. depressor Mandibulae. It obviously acts as a crude tympanic membrane. (fig. 3).

An even more interesting transition from V.Q. to V.T. is found in agamid and iguanid lizards (Smith, M., 1938):

(a) In *Chameleon* and *Holbrookia,* the columella auris terminates in a knob, which is attached to the posterior surface of the quadrate bone. This is V.Q. (See fig. 4A.)

(b) In *Lyriocephalus* etc., there is no tympanic membrane, its place being covered not only by skin but also by muscle (Depressor mandibulae). The middle ear cavity is rudimentary and may be absent.

(c) In *Otocryptis,* there is no visible tympanum, the membrane being covered by skin. On stripping the skin away the membrane can be seen, together with the attached extra-columella cartilage. The middle ear cavity is fairly large—the stapes crossing it is exposed but never completely free. These animals have not quite perfected the V.T. mechanism (fig. 4B).

(d) In *Gecko,* the stapes is a fragile spicule of bone only held by a delicate strand of supporting tissue. A perfect trilaminate drum is present (fig. 4C).

This transition crystallises the issue between the two theories. On orthodox theory it is retrogressive and degenerative, i.e. from a pristine perfect air-sensitive ear V.O., to a more modern degenerate form V.Q. On functional theory the change is from a pristine crude V.Q., by an evolutionary improvement, to a modern perfected V.T. We shall also encounter later a transitional mechanism between V.Q. and V.O. No examples of this mechanism (which I call Vestibulo-Mandibular V.M.) are extant, but it represents an itnermediate stage between the V.Q. of the pre-mammalian reptiles and the V.O. of their descendants the mammals.

The Testimony of Palaeontology

Now let us review the anatomical facts which have been supplied by palaeontology and which must inevitably provide the basis for any more theoretical speculations. The stapes first appears as such in the early Stegocephalia—e.g. *Eogryrinus.* These animals were so primitive that they still possessed the lateral line organ. This —the true hearing organ of the fish—is not found in modern Amphibia (except in larval forms). *Eogyrinus* furthermore resembled the fish in that it possessed no oval window; nevertheless, it has been generally assumed that a tympanic membrane

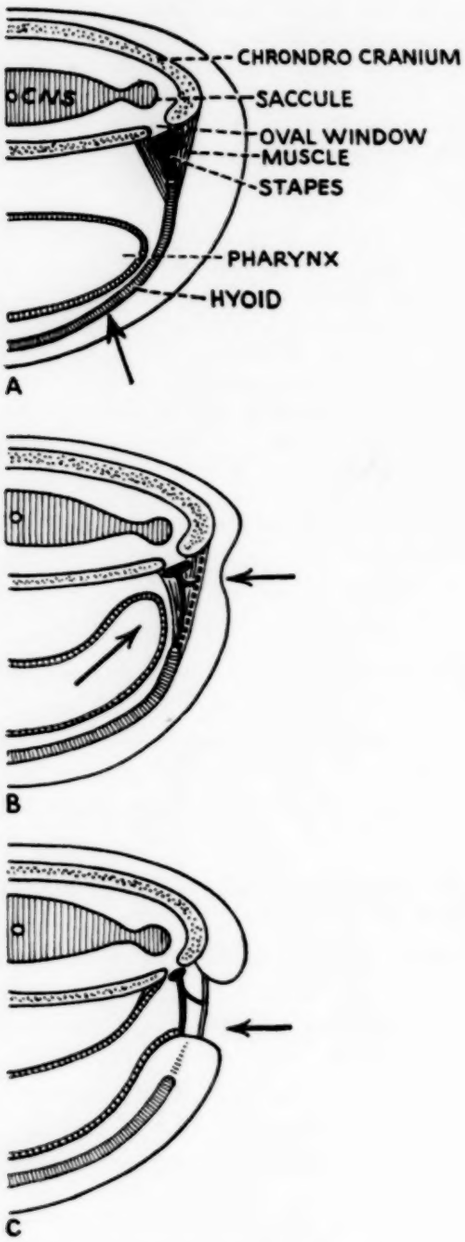

FIG. 3. The transition from vestibulo hyoid to vestibulo tympanic hearing. A: *Stage 1.* Still present in amphisbaenids. The stapes is a solid conical mass overlain by muscles and articulating with the hyoid. This is essentially a bone conducting device as used by the prostrate burrowing amphisbaenids, but it is potentially air sensitive. B: *Stage 2.* The air sensitive potentialities of V.H. are beginning to materialise. The stapes, although still connected to the hyoid, now sends—in addition—a process to a plaque of fibrous tissue incorporated in the overlying muscles. Air-borne vibrations energise the stapes either via the floor of the mouth and the hyoid or via the fibrous plaque. This arrangement is found in Sphenodon today. C: *Stage 3.* The perfected air sensitive device of the Bird. The muscles overlying the fibrous plaque have receded. The pharynx sends a pouch up to the stapes. The connection with the hyoid becomes tenuous. Arrows show direction of sound conduction.

was housed in the otic notch. How valid is this picture? In that respect figure 5 should be examined. It represents the skull of a modern lizard. It too shows a slender stapes projecting out towards the surface. It would be easy to draw around this stapes a tympanic cavity and membrane just as has been done with the Stegocephalia. But *Lyriocephalus* is a modern lizard and we know that it possesses neither tympanic membrane nor cavity. Nor, in the writer's opinion, did *Eogyrinus.* It is true that the stegocephalian stapes appears to end freely in the centre of the otic notch but there may well have been a cartilaginous extra columella connecting it with the edge of the notch. This would make it absolutely identical with the vestibulo-squamosal mechanism of the modern urodele—a bone-conducting device. The allocation of an air-sensitive middle ear to *Eogyrinus* has in fact nothing to commend it.

An animal still using the lateral line system characteristic of an aquatic environment would be most unlikely to have developed such a highly specialised middle ear so characteristic of air-borne sound. Furthermore, it can be shown that in the absence of an oval window, air-borne hearing is practically impossible. A tympanic membrane would have been useless to *Eogyrinus.* Bone-conducted hearing of a sort, however, is quite feasible even in the absence of an oval window and would almost certainly be the method of choice for an animal which only occasionally ventured onto land and which even than was completely prostrate. It is unjustifiable to decide that the stapes terminated in the *centre* of the otic notch unless and until we can speak with some certainty about the possibility of a cartilaginous extra columella. Unfortunately, such a structure is unlikely to leave recognisable traces, and in the absence of such direct evidence we must look for indirect evidence. Ac-

tually, palaeontology does provide us with abundant evidence of an indirect nature which speaks emphatically against the stegocephalian tympanic membrane as the following argument will show.

Let us assume for a moment that an air-sensitive ear was in fact present in *Eogyrinus*. Presumably this would have been available for the subsequent stem reptiles and, as they were in the main erect quadrupedal, it would have been an immensely valuable asset, much superior to any of the bone-conducting devices I have described. Once the head is lifted off the ground, bone-conducted hearing becomes useless. We are entitled to expect that this master patent of air-sensitive hearing, if it ever existed, would still be found in the reptiles and indeed would steadily improve to meet the increasing demands of the evolutionary struggle. And yet apparently we meet just the opposite. This invaluable tympanic membrane is apparently driven from pillar to post. In the cotylosaurs, figure 6, the rigid ideal otic notch has disappeared. The site of the hypothetical drum of the cotylosaur is indeed rarely discussed, but those writers who persist in believing it existed at all are compelled to envisage it somewhere behind the jaw closely associated with the muscles of mastication where presumably it would be dragged and pulled with every snap of the jaw. This is indeed a sad descent from a secure otic notch, but worse is to follow for it seems quite clear that the Pelycosaurs—the dominant beasts of their time—had completely lost this priceless organ and had in its place acquired a V.Q. mechanism which, whatever value it might have been for a prostrate animal, would be terribly inefficient for an erect quadruped. Dabelow (1928) says, for example:

"Bei Dicynoden der Stapes liegt geradezu wie ein Stethoskop dem Quadratum und somit der Wand des Kiefergelenkes an, und man sollte vermuten, das es der Cochlea also dem 'eigentlichen' Ohre nichts weiter übermitteln kann als die Gerausche des Kiefergelenkes, da das massige, mit dem Quadratojugale verbundene Quadratum kaum durch Schallwellen in Schwingung

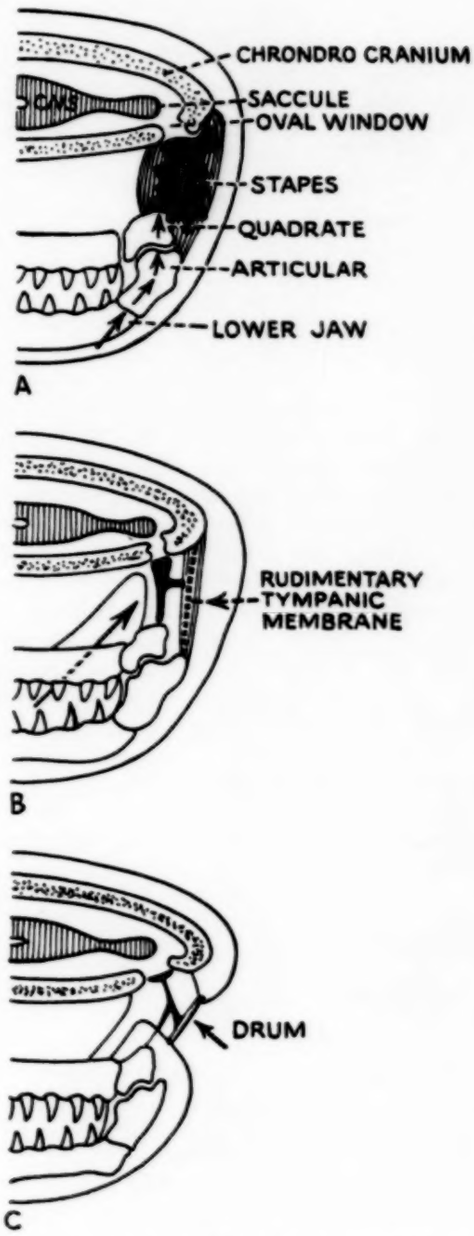

FIG. 4. The transition from vestibulo quadrate to vestibulo tympanic hearing. (A) Vestibulo Quadrate—hearing via lower jaw. The stapes is massive and closely articulated with the quadrate. Found in many modern reptiles and also in the Premammalia. Compare figure 7 and figure 2B. (B) Transitional stage seen in many lizards today. The stapes has become less massive and contacts a rudimentary tympanic membrane which develops as a fibrous plaque in the overlying masticatory muscles. The pharynx sends out a diverticulum. Compare figure 5. (C) Perfect vestibulo tympanic hearing found in many lizards such as Gecko. The stapes is air-borne—the pharyngeal diverticulum produces a tympanic cavity and reaches the fibrous plaque. The muscles recede and a trilaminate drumskin is formed. This mechanism closely resembles the avian V.T., but it would be wrong to regard them as identical.

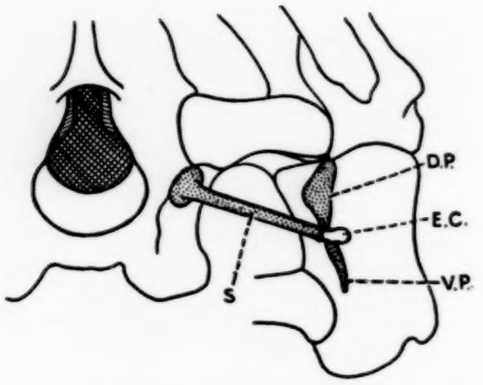

FIG. 5. Hind view of skull of *Lyriocephalus scutatus*. This modern lizard has no ear drum and no middle ear cavity. The stapes S is a slender stylus projecting outwards from the oval window. Its dorsal process (DP) and ventral process (VP) are attached to the quadrate whilst a small cartilaginous extra columella (EC) ends in the superjacent muscles. This arrangement should be compared with figures 1A and 1B. It illustrates the danger of postulating soft tissue structures such as a tympanic membrane and cavity solely from the appearance of the bony skeleton. (From "evolutionary changes in the middle ear of certain Agamid and Iguanid lizards," by Malcolm A. Smith, Proc. Zool. Soc. Lond., Ser. B, 108, Part 3, 1938, by permission.)

versetzt werden kann. Mann muss also wohl auch aus diesem Grunde annehmen, das den premammalischen Reptilien ein Trommelfell noch fehlte."

This, of course, is not entirely true. The animal could hear by placing its jaw on the ground or against a tree trunk but in its ordinary stance—with head free—it was DEAF. This conclusion, following the assumption of adequate hearing in the Stegocephalia, is so repugnant that it is not surprising that some authorities refuse to face it. Westoll (1943)—as noted above—insists that a tympanic cavity was present throughout but the weight of authoritative opinion is all against him.

Dabelow's opinion has already been noted.

Watson (1953) says:

1. "In *Ophiacodon* . . . it is obvious there can have been no tympanic membrane."
2. ". . . there is no place where a tympanic membrane could be placed in *Dimetrodon*."
3. "The *Dimetrodon* condition is typical of early mammal-like reptiles in general."
4. "In *Ulemosaurus* it is evident that the stapes can never have come into contact with a tympanic membrane."

Figure 7 shows a series of pre-mammalian skulls. In each case the stapes is a massive bone well designed to act as a bone-conductor. It could not possibly respond adequately to the feeble flapping of a tympanic membrane.

In some pelycosaurs and most therapsids the angular shows a notch which is believed by some writers to have housed the tympanic membrane. Romer and Price (1940) discussing this problem say:

"It has been assumed by some writers that this notch lodged all or part of the tympanic membrane. After careful consideration of the topographic situation, however, it is concluded that this interpretation is quite untenable.

"1. It is obvious that the presence of a tympanum here implies the development of a new drum and middle-ear cavity not homologous with those seen in other reptiles. There can have been no gradual migration of the drum from a more dorsal site to that seen here, for the way is blocked by the depressor mandibulae muscle which would still have been functional in pely-

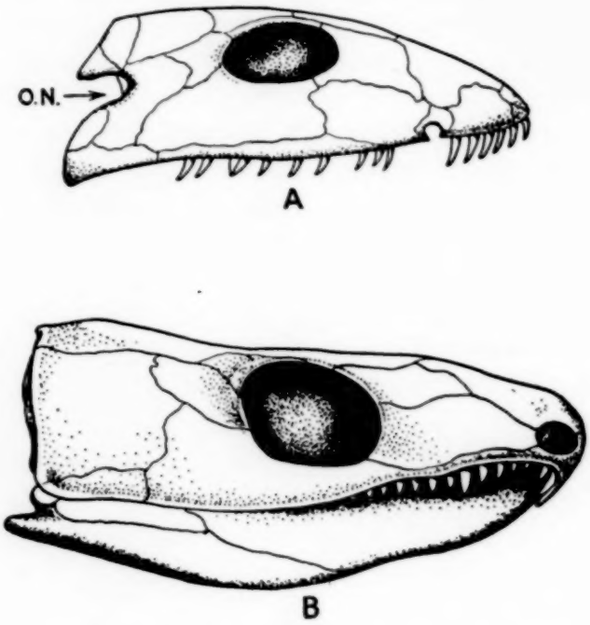

FIG. 6. The disappearance of the otic notch. A: Ichthyostega—a very early amphibian. B: Captorhinus—an early reptile. If the notch really housed a tympanic membrane in the amphibia, it would not have disappeared in the reptiles. The ear is far too important to be thus jettisoned. (See text.)

cosaurs and which is intermediate in positions between old and supposedly new drum areas. There must therefore have been an abrupt change—a 'Saltation' (within the pelycosaur group) of a most discontinuous sort.

"2. While in some instances the notch appears to form a suitable margin for a tympanic membrane, in others this is not the case. The area beneath the upper part of the notch underlain by the main body of the angular is very shallow. In some cases, such as in a jaw of *D. grandis,* the shape of the angular flange is such that the drum must have been confined in this dorsal area. The notch margins and the surface beneath it are only a few millimetres apart and this hardly allows enough room for an air pocket beneath the drum, to say nothing of the lack of space for the insertion of an extra-columellar connection.

"3. A position of the drum here requires the presence of an extra-columellar apparatus of unbelievable complexity. The end of the ossified stapes is inside the quadrate, somewhat higher than the supposed drum, and directed backwards and outward. To reach the notch region we must suppose the preesnce in cartilage of a fishhook shaped apparatus turned 45 degrees outwards, which first turned ventrally 90 degrees to pass beneath the articular region, then outward 90 degrees, and finally upward 90 degrees to reach the supposed drum. On both functional and purely morphological grounds this is difficult to believe.

"4. The area beneath the notch is normally occupied by the pterygoidens muscles, and to allow for middle-ear development here it must be assumed that this muscle mass is unusually reduced.

"5. If the notch contained the drum, it should retain a posterior position in all synapsids. In cynodonts, such as Cynognathus, however, the notch moves to a point far forward along the jaw, a position phylogenetically improbable for a drum, and considering the marked shifts in position when the jaw is opened and closed, mechanically impossible when stapedial attachments are considered."

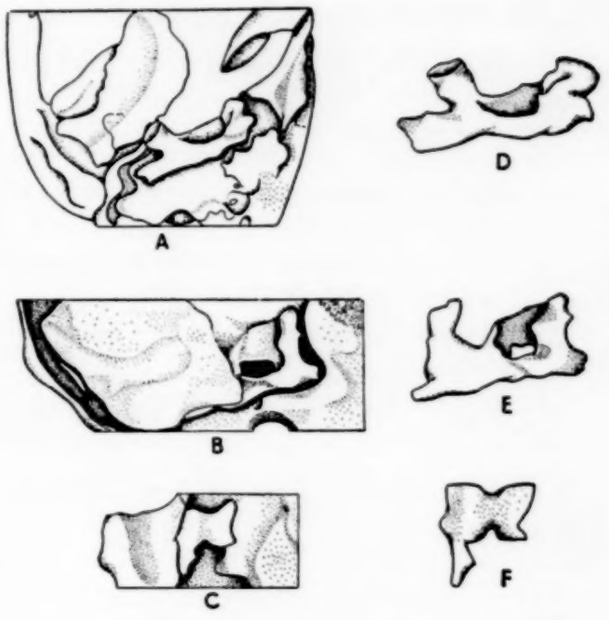

FIG. 7. The therapsid stapes. Ventral view of right tympanic regions and stapes of selected therapsids. The stapes is drawn separately in each case on the right.

A: Therocephalian.
B: Gorgonopsian.
C: Anomodont.

Note the relatively enormous size of the stapes. In each case the bone is so firmly locked to adjacent bone—especially the quadrate—that a tympanic membrane would have been entirely ineffective. There can be no doubt that these reptiles heard by bone conduction. Air sensitive hearing only developed in their mammalian descendents. Consequently we deduce that it was *not* present in their amphibian ancestors. Such a vitally important sense organ could not temporarily disappear. See text. (After E. C. Olson, "Origin of mammals," 1944, Spec. pap. Geol. Soc. Amer., No. 55, p. 40.)

It cannot be doubted therefore that even if a tympanic membrane existed in the Stegocephalia, it had disappeared in the cotylosaurs and pelycosaurs. This "disappearance" of the tympanic membrane must arouse the gravest doubt. It would be no more absurd to predicate that for some prolonged period— long after a perfect eye had been evolved—the dominant groups went blind and then subsequently rediscovered the secret of vision. The logic of the situation is therefore inescapable. If the Amphibia possessed a tympanic membrane it is impossible on functional grounds to believe that their reptilian successors would have lost it. But their successors did *not* possess a tympanic membrane. Therefore the Amphibia cannot have had one to pass on. We have already seen that there is no convincing evidence to support the Stegocephalian tympanic membrane and the whole situation is clarified once we agree to reject it.

The Functional Theory

I submit that an adequate theory of the evolution of the middle ear must fulfil the following criteria:

1. It must harmonise with the available anatomical facts.
2. It must harmonise with the functional facts.
3. It must harmonise with the morphological facts.
4. It must harmonise with the ontogenetic facts.

In what follows I attempt to outline such a theory.

The Earliest Amphibian Middle Ear

Consider a Devonian amphibian—a large beast lying prostrate semisubmerged in water or mud, assailed by sound from above *and below*. Not least among the problems confronting this animal in its change of environment was the need for a different auditory mechanism since the lateral line system so suited to an aquative environment would be much less efficient in air. We need not discuss the reasons for this; it will suffice to note that this system rapidly disappeared, being replaced by an auditory system based on the organ of equilibrium—the labyrinth. This transformation involved no act of creation—nor need we invoke orthogenesis or macro-evolution to explain it. The "inner ear" developed in the labyrinth simply because in the last analysis sound is a form of pressure just as gravity is. Thus the neuro-epithelium of the static labyrinth, being sensitive to gravity, was also sensitive—even if only in a rudimentary way—to the impact of sound waves. It is not difficult to envisage how a portion of the saccular neuro-epithelium would become specialised to respond to sound waves as we see in modern amphibia. The middle ear, following the proposed definition, would consist of all those structures capable of transmitting acoustic vibrations to the inner ear. In a sense, this might be said to include the whole body but canalisation of the conducting pathways would no doubt occur fairly rapidly because of the great importance of the auditory function. The precise route adopted was no matter of chance. On purely physical grounds we should expect it to include the hyomandibular since that bone was attached to the chondrocranium precisely over the labyrinth, so that from the very beginning it was the main route for all incoming vibrations from the periphery. The hyomandibular route was favoured, however, because of another "biological factor." The complex fish skull was tending to fuse and to simplify. In particular, the pterygo-maxillary complex was joining up with the chondro-cranium so that the suspensory mechanism—the hyomandibular—was becoming redundant. It is not surprising therefore that—losing its suspensory function—the hyomandibular took on an acoustic conductive function. Now Westoll (1943) has shown that in addition to its ventral process attached to the auditory capsule, the hyomandibular possessed four other processes.

1. Dorsal to the dermal skull bones.
2. Opercular to the operculum covering the gill cleft.
3. Hyoid to the hyoid bone.
4. Quadrate to the quadrate bone.

Vibrations from the substrate would travel along the lower jaw and also the hyoid to the quadrate and hyoid processes respectively to reach the ventral process and so the auditory capsule. If the animal was semi-submerged the dorsal and opercular processes would also pick up water or mud-borne vibrations. It is true that air-borne sound waves would impinge on the skull if the animal was not submerged. *Elementary physics, however, teaches us that such air-borne vibrations would be almost entirely reflected off the body surface.* Substrate vibrations would be received at least 100 times more efficiently than air-borne vibrations. Thus the earliest amphibia developed bone-conducting middle ears because there was simply no

physical or biological reason for them to develop air-sensitive ears since all significant noises were transmitted by and received from the substrate more efficiently than from the air. Furthermore, this bone-conducting mechanism was already present, so to speak, by accident and needed comparatively little modification to go into action. Those familiar with the infinite plasticity of the evolutionary process will not be disposed to deny that every possible route was probably utilised by some group or other of these developing amphibia and that specialisation and differentiation took place as time went on. In each case, the ventral process persisted as the footplate of the stapes. The labyrinthodonts seem to have combined this with the dorsal process to form the V.Sq. mechanism. Presumably the urodeles are descended from that group.

The Early Reptilian Middle Ear

Other groups presumably specialised in the quadrate and hyoid routes respectively. The resulting V.Q. and V.H. would be more fitted for life *on the* land surface than V.Sq. which befits the semi-submerged existence so that those amphibia which happened to develop V.Q. and V.H. would be more successful in invading dry land to give rise to the reptiles. We deduce therefore that the cotylosaurs (using this term to include the precursors of all other reptilian groups) utilised V.Q. and/or V.H. In the subsequent radiation of the reptiles some groups specialised in V.Q., others in V.H. The former mechanism was utilised by the pelycosaurs and also presumably by the pro-squamata since such a condition is still found today in Ophidia and many Lacertilia. The limitations of this mechanism are obvious. The animal is deaf unless the jaw is on the ground, or resting against a tree trunk or other solid object. And yet the pelycosaurs were a very successful radiation. That is quite true and can only be explained by the fact that they had no serious rivals. For a long time—apart from insects—there were only amphibia using V.Sq. and presumably other reptiles using V.H.—both mechanisms basically just as crude as V.Q. of the pelycosaurs. Thus the latter flourished, and their very success, as we shall see later, contributed to their ultimate downfall.

Mechanical and Geometrical Considerations

It is necessary at this point to say a few words about the relative efficiency of different mechanisms under various circumstances. On page 230 I said that a large prostrate amphibian would receive substrate vibrations more efficiently than air-borne vibrations. On the other hand, I am now saying that the air-sensitive ear is a more efficient mechanism than the bone-conducting mechanism. These statements may appear contradictory but are in fact not so. We have to allow for the nature of the mechanism itself and also for the source of the vibrations it detects. We may regard the large amphibian as an amorphous mass with a specific gravity much nearer to that of mud than to air. In that case it is quite true that sound vibrations in the substrate will freely enter the mass whereas air-borne sound will bounce off. That is a physical fact. In this example we also have the biological fact that the sounds which would be significant to such an animal would be mainly produced in and transmitted by the substrate. They would only emerge into the air to a limited extent. Of course, *if this animal possessed an air-sensitive ear,* it would pick up air-borne sound very efficiently—at the expense of comparative deafness to earth-borne sound just as the modern crocodile. That, however, begs the question. We know that at some period sufficiently remote the drum did not exist—the ainmal was little removed from the fish—and the question is—"How would the auditory pathways develop in such an undifferentiated mass?" All the evidence points to the bone-conducting route. The position is different in the

erect quadrupedal pelycosaurs. The substrate would still transmit the sound of their tread—but the air would be carrying far more of the sounds they made in their predatory, procreative and other activities. A bone-conductor to such animals would be of little value. It could only detect substrate vibrations if the animal deliberately placed its head on the ground—or a tree trunk.

What modifications are necessary to convert a bone-conducting mechanism into an air-sensitive mechanism? A detailed discussion of this question would be out of place here, but we may find our answer best by examining a typical ear. Two points stand out. First, the moving parts are made as light and free as possible, and second, the aerial energy is collected on a large "tympanic membrane" and is concentrated on a small oval window. This is a sort of lever action comparable with the electrician's transformer for matching impedances. On the other hand, in bone-conduction the mass of the conducting mechanism is irrelevant. We have seen

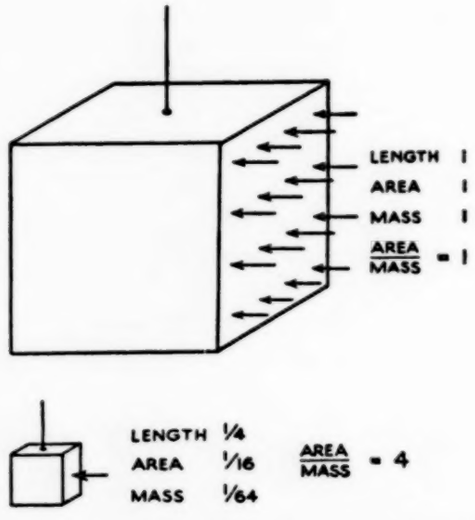

FIG. 8. A bio-physical principle. The ratio of area to mass increases as the object shrinks in size. Thus a small animal is more sensitive to air-borne sound than a large animal. Functional theory suggests that air-sensitive ears developed independently in Anura, Archosaurs, Squamata and Mammalia and that in each case the transition from preceding bone-conducted hearing took place when the animals were defeated and were consequently tiny.

that the pelycosaurian stapes was so massive that it could not possibly be energised by any tympanic membrane which could be feasibly housed in its skull; on the other hand, it was quite adequate as a bone-conductor. But figure 8 reveals an interesting and relevant geometrical fact—namely that as a body decreases in size, the ratio of area to weight *increases*. In other words, a small animal will be more sensitive to air-borne sound than a large animal. This has, in the writer's opinion, been one of the most important factors in determining the emergence of air-borne hearing. The evidence indicates that such a mechanism emerged independently in at least four different groups and in each case this happened not when the beasts were of large size, but when they were quite small. It seems clear that the pelycosaurs were the large animals of their time whilst side by side with them were the more obscure types—including archosaurs and Rhynchocephalia which utilised V.H. as *Sphenodon* does today.

First Appearance of Air-Sensitive Hearing

V.H. has so far been described as a bone-conducting mechanism—but it is *also* in a way a crude air-sensitive mechanism. We may regard the whole floor of the mouth as a large—if rudimentary—tympanic membrane, the hyoid bone corresponding functionally to the mammalian malleus. The enormous and, so to speak, accidental virtue of this dual purpose mechanism was that it was not *earth bound*. Its possessor could hear even when standing upright. It is impossible to resist the conclusion that the upright bipedal stance of the archosaurs was rendered possible—and indeed promoted—by the happy chance that they had originally specialised in V.H. The transition from V.H. to V.T. requires no great feat of imagination and we can well believe that this transition may have given the dinosaurs the advantage which enabled

them to annihilate the sluggish pre-mammalia almost entirely.

Later Changes in Amphibia

We now resume the story of the amphibia semi-submerged—utilising V.Sq.—left behind by their more fortunate competitors whose V.H. and V.Q. had enabled them to clamber on to land. The following morphological data must be accounted for by any adequate theory, namely that each of the two main groups of amphibia possesses a *double* mechanism.

The urodeles possess V.Sq. and V.S.
The Salientia possess V.Sq. and V.T.

It is immediately apparent that these mechanisms conform closely to the habits of the animals in question. V.Sq. corresponds to the semi-submerged state, V.S. to the elevated head and V.T. to the jumping animal. Indeed the correspondence is much closer than the above bare statement indicates. Amongst urodeles, for example, the more aquatic favour the V.Sq. mechanism whilst V.S. is favoured by the more terrestrial types. Similarly —amongst Salientia—the toads that walk rather than jump favour V.S. Indeed in some, such as *Bombinator*, there is no tympanic membrane at all whereas in the frogs —the real jumpers—the drum mechanism is exquisitely developed and the fore-paw mechanism is reduced. Classical theory pays little or no attention to this remarkable functional correspondence. As in the case of the Lizards, pre-occupation with air-borne hearing leads to the assumption that the air-sensitive ear of the Salientia is directly inherited from the air-sensitive ear of the Stegocephalia. The V.Sq. and V.S. mechanisms—so far as they are recognised at all—are dismissed as irrelevant or degenerate. On functional grounds this is quite unacceptable. The function of the various amphibian middle ears is not only strikingly in accord with the modern requirements, but it is exactly what could be predicted from our knowledge of the prehistoric course of events. Furthermore, the modern Salientia cannot have inherited a tympanic membrane from the Stegocephalia since the latter did not possess one to bequeath.

The modern Amphibia are—in the writer's opinion—the descendants of those early amphibia that had specialised in V.Sq. These would have remained in their semi-submerged condition, however, if they had not succeeded in evolving a new auditory conducting route suited to dry land. V.S. is the obvious route. It was the next most direct route to the hyomandibular and was utilised because the latter was no longer available, having been used up in V.Sq. Readers sceptical of this suggestion should know that many deaf children can be taught to recognise sounds by feeling a vibrating surface with the finger-tips. Having emerged onto dry land by means of V.S., some of these amphibia went on to develop jumping proclivities and this was made possible by the evolution of an air-sensitive tympanic membrane which rendered them independent of the substrate vibrations. On this theory, V.Sq. and V.S.—so far from being degenerate—were logical and inevitable stages in the evolution of the auditory function.

The Mammalian Mechanism

Next we take up the story of the pre-mammalians. At the height of their power they were large brutes and for that very reason, their stapes was so bulky that it was quite insensitive to air-borne sound. The necessary physical conditions were not present for the development of air-sensitive hearing, and that master patent was instead evolved by the obscure—and presumably small—archosaurs. Subsequently the tables were turned—the dinosaurs flourished—the pre-mammalia receded into obscurity. It is generally believed that by the time the reptilian-mammalian transition began to take place, the creatures were quite tiny—no bigger than the modern lizard. It might have been expected therefore that air-sensitive hear-

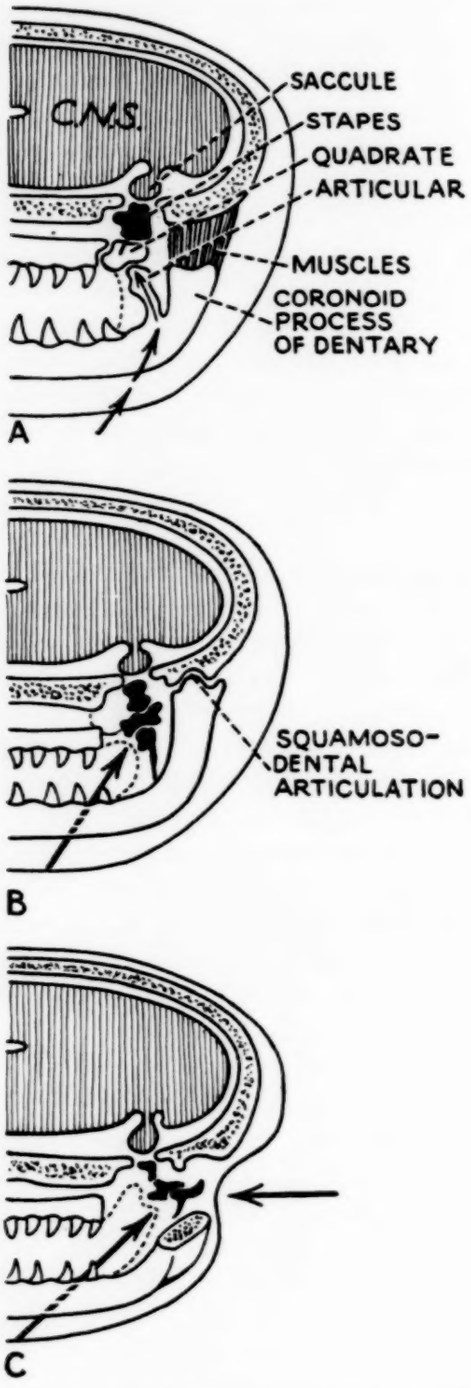

FIG. 9. The transition from Vestibulo Quadrate to Vestibulo Ossicular hearing. A: *Stage 1.* The C.N.S. towers above the ear and bellies out external to it. The oval window sinks inwards. The jaw still hinges at the articulo-quadrate joint which has become delicate. Note the massive coronoid process of the dentary. Vestibulo Quadrate hearing in Cynognathus. B: *Stage 2.* The dentary has reached the squamosal to form the mammalian joint. The reptilian joint is superseded. The articular and the quadrate fall into a disuse atrophy which makes them all the more suitable for auditory purposes. This arrangement is mechanically very similar to V.H. since the ossicular chain lies under the mucous membrane of the buccal cavity. The mechanism is primarily bone conducting but—like V.H.—it is crudely sensitive to air-borne sound (perhaps especially when the mouth was open). Vestibulo Mandibular hearing in earliest mammals. C: *Stage 3.* The air-sensitive potentialities of V.M. materialise. The three ossicles continue to shrink. The outer ear canal and the Eustachian tube begin to form, and as they approximate, the articular is trapped in the intervening connective tissue and becomes the malleus. (N.B. The ramus of the mandible has been excised to show the ossicles.)

ing would develop in the therapsids by a simple transition from V.Q. to V.T. How then are we to explain this fantastic incorporation of the articulo-quadrate complex into the auditory conductive chain? One part of the answer has been provided by Dabelow (1928) who points out that owing to the development of the central nervous system in the therapsids the brain bulged outwards so that the oval window sank inwards (fig. 9). Consequently the stapes—attached to the quadrate—was also carried inwards and could not easily reach the surface. The other factor is the transformation of the jaw joint. The reptilian articulo-quadrate joint was being superseded by the mammalian squamoso-dental (temporo-mandibular) joint. We need not discuss in detail the factors that determined this particular transformation. Partly they were mechanical, being evoked by the increasing importance of the masticatory function. The mouth of a fish is an engulfing maw—its main function being to trap and kill prey. The reptiles, however, were using the mouth for grinding and masticating food. This no doubt necessitated a different arrangement of muscles, and perhaps a shorter lever action. Furthermore, the relatively enormous increase in size of the central nervous system was radically altering the contours of the skull as a whole, making it high and narrow. The increasing importance of the temporal and other masticatory muscles accounts for the remarkable overgrowth of the dentary. *Cynog-*

nathus—figure 10—shows a comparatively late stage in this transformation. The dentary is enormous—the articular is slender, but even so the jaw still hinges on the articular.

Figure 9 shows the subsequent stages. The squamoso-dental joint has taken over, leaving a quite small articulo-quadrate combination which no doubt would have gone into a disuse atrophy except for the fact that it was also subserving an auditory function. This state of affairs is functionally identical with the transformation of the piscine hyomandibular into the Amphibian auditory stapes. In these late Therapsids, the articulo-quadrate complex obviously lay in fairly close apposition to the buccal mucous membrane where it would vibrate to *air-borne* sound if the animal opened its mouth. I have labelled this mechanism V.M. Vestibulo-Mandibular. Its mode of action is very reminiscent of V.H. and its supreme importance lay in that fact. The V.Q. bone-conducting mechanism—by this addition of the atrophic articulo-quadrate complex—became a crude air-sensitive mechanism and brought with it the emancipation of the mammals.

The virtues of this explanation are obvious. It features a steady progressive *improvement* in the auditory function of the animal pari passu with the physical and biological changes which were then occurring. The incorporation of the two extra ossicles is shown to be logical, necessary and advantageous to the animal. The early pre-mammalia started with V.Q. which—because of their erect posture—was almost useless. V.M. is also manifestly a crude device—*but it is* better than V.Q.—and furthermore it was capable of refinement into the exquisite V.O. Thus the transition V.Q. → V.M. → V.O. is a progressive improvement such as we expect to find in the evolution of a premier sense organ such as the ear. Orthodox theory, on the contrary, assuming as it does a perfect air-sensitive middle ear in the therapsids, can find no valid explanation for this transition to a triple

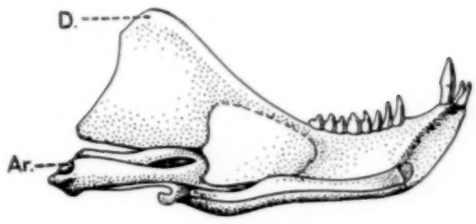

FIG. 10. Inner view of lower jaw of Cynognathus. The jaw still rotates on the articulo-quadrate joint, but the Dentary is massive and upstanding. It is easy to see how the Squamoso-Dental joint came into being. The articular, lying under the buccal mucosa and falling into a disuse atrophy, would become increasingly sensitive to air-borne sound. (From "Vertebrate palaeontology," A. S. Romer, p. 284.)

ossicle system. Once more we face the paradox of temporary deafness for it is impossible by any feat of imagination to interpolate an obsolescent jaw joint into a "perfect middle ear" without some temporary sacrifice to function. Nor, so far as we know, would the end result justify such an interpolation. It is sometimes assumed that the triple ossicle system is in some respects superior to the single ossicle system. That is a grave error. There is no evidence to that effect and we must conclude that the two extra ossicles were co-opted in order to make possible the transition from crude bone-conducting V.Q. hearing to air-conducting hearing under the special circumstances which I have outlined. This transition took place—not as Watson suggests as a casual by-product of mechanical changes in the skull—but as a result of the complex interplay of many biological factors amongst which must be counted not least the vital importance of the auditory function itself in the struggle for survival.

The Middle Ear of the Dinosaurs

Apart from one reference by van Huene, no one has ever described the middle ear of the dinosaurs. Lull & Wright (1942) say:

"It is regrettable that the actual bony stapes has apparently never been recorded in dinosaurs."

This extraordinary state of affairs has never received due recognition, especially when we consider that skulls four feet and more in length have been examined. In comparison with the pelycosaurs such skulls might be expected to shew stapes four or five inches long—provided of course that the auditory mechanisms were the same in each case. We must deduce therefore that the auditory mechanisms were *not* the same and there are two possibilities.

First, the dinosaurs may not have possessed a stapes at all. This, whilst theoretically not impossible, is quite repugnant. As in the case of the pelycosaurs, the dinosaurs were the ruling animals for 50 million years or more. It is impossible to believe that this vast radiation could have been uniformly deaf, not even boasting the crude bone-conducting devices of their defeated rivals.

The alternative hypothesis is that a stapes *was* present but that it was so tiny and so freely suspended in the middle ear cavity that it has not survived the forces of fossilisation. This is the writer's own opinion and it arises naturally from the functional theory. We have argued that the bipedal stance of the archosaurs was rendered possible by their possession of air-sensitive ears. The precursors of the dinosaurs were the thecodonts described as *small* reptiles with about the range of size seen in modern lizards. It is not unreasonable to argue that the transition from V.H to V.T. would arise *somewhere* in this group. We know that they gave rise to the birds and to the pterosaurs and it is difficult to believe that they could have taken to the air unless and until they had acquired an efficient air-sensitive ear. The position may be summarised as follows:

1. The Vestibulo-Hyoid mechanism was certainly utilised by *some* groups of primitive animals, e.g. Rhynchocephalia.
2. The possessors of V.H. would be able to stand up without becoming deaf.
3. The transition from V.H. to V.O. would occur most readily in small animals.
4. V.T. would give its possessor an enormous advantage over its earth-bound rivals.
5. The characteristic feature of air-sensitive stapes is that it is extremely fragile and so would not survive fossilisation.

These are all "functional" propositions. They are independent of each other and yet they harmonise perfectly with each other and with the palaeontological data. In particular, they explain the contrast between the massive pelycosaurian stapes and the ephemeral dinosaurian stapes. The mystery of the missing dinosaur stapes is thus explained and leads to the original conclusion that they were the first animals to perfect an air-sensitive ear.

The Lacertilian Middle Ear

Little need be said on this point. The issue between the two theories is quite clear—evolution from V.Q. to V.O. or degeneration from V.O. to V.Q.

The Testimony of Ontogeny

The Relation of the Anuran Columella to the Sauropsid Columella

Orthodox pre-occupation with the air-sensitive ear has led to the uncritical assumption that all such ears are homologous—despite very real anatomical differences. Air-sensitive ears are found in Amphibia, reptiles, birds and mammals and it seemed reasonable to assume that they were all descended from a similar organ in the common ancestor—the stegocephalian. This simple hypothesis—so plausible at first sight—turns out, however, to be quite untenable.

We have seen that there is no evidence for—and much evidence against—the stegocephalian tympanic membrane. We now turn to the other end of the genealogical tree—to the assumption that modern air-sensitive ears are homologous. This also turns out to be without foundation. It is obvious that there is nothing in the sauropsid middle ear corresponding to the mammalian incus and malleus and detailed examination of the intra-tympanic muscles, ligaments and of the drum itself

reveals such diversity that it is impossible to homologise them. Nevertheless, there has always been one structure which has been assumed to be constant throughout the terrestrial vertebrate middle ear—namely, the stapes. The "constancy" of the stapes is indeed a fundamental assumption in orthodox theory and provides the basis of a crucial test since the functional theory speaks emphatically against it.

According to the functional theory, the foot-plate of the stapes is derived from the ventral process of the hyomandibular—*in most animals*. Whereas, however, in urodeles, the remainder of the bone arises from the dorsal process, in birds it arises from the hyoid process and in mammals and lizards from the quadrate process. These differences, however, are perhaps trivial and could be covered by the statement that the stapes arises from the hyoid anlage. In the case of the anuran stapes, however, the issue is quite clear cut since functional theory declares that the anuran stapes cannot have arisen from the hyomandibular at all. Let us return to the Stegocephalia at the stage when they were developing the V.S. mechanism which enabled them to raise the head. Whence did the opercular cartilage arise? Surely not from the hyomandibular since the latter was still in use for V.Sq. (as it is in urodeles today). The musculus operculari is only a specialised portion of the levator scapulae which is inserted into the skull. It is not unreasonable to argue that the opercular cartilage is also a part of the skull—literally shaken loose in response to functional demands. This speculation received striking confirmation from ontogeny. Authorities are quite unanimous in deriving the opercular cartilage in the developing amphibian from the chondrocranium, and not from the hyoid anlage. Let us pursue this argument further—to the emergence of the anuran tympanic membrane. I have suggested that this was almost certainly a later development. In accordance with the above-mentioned geometrical principle, I suggest that the anuran tympanic membrane did not develop until the proto-batrachians were quite tiny animals—defeated no doubt by the reptiles. Whence did the anuran stapes arise? According to orthodox theory from the hyomandibular. According to functional theory it could not have done so since the hyomandibular had been used up in V.Sq. and was not even available for V.S. which must have preceded V.T. This issue is one which can be put to the test in the study of ontogeny. The hyoid origin of the sauropsid and mammalian stapes can be demonstrated without difficulty. In the anuran, however, it has been known for over fifty years that the stapes has an entirely different origin.

Gaupp (1898), the greatest authority on the frog, wrote as follows:

> "Recently Villy, Killian and myself have investigated the development of the sound conducting apparatus in the salientia. We have reached results which in all essentials agree—namely, that ontogenetically neither the operculum nor the columella has anything at all to do with the hyoid arch and that the auditory capsule is the foundation out of which their structure develops."

It is quite impossible to reconcile this fact with a belief in the homology of the anuran and sauropsid stapes. It is perhaps not surprising therefore that protagonists of the orthodox theory ignore this ontological evidence. Westoll (1943), for example, says:

> "Alone among known tetrapods the amphibia have the fenestra ovalis partly plugged by two cartilages each presumably part of processus ventralis (of the hyomandibular)." In a footnote he adds: "According to several investigators the operculum may be derived from the wall of the otic capsule. The nature of the 'stapes' of the Stegocephalia supports Goodrich's view that it is of hyoid arch origin."

The issue—being of such vital importance—seemed worthy of reinvestigation and the ontogeny of the anuran auditory mechanism was therefore recently carefully investigated at the University of Liverpool by Miss Tinne (unpublished). The following quotations from her work are relevant:

1. "The auditory ossicles arise from two entirely different sources. From my own observations the Operculum and the Otostapes (i.e. footplate of columella) are unquestionably of Otic origin. The middle part of the Columella (Mediostapes) appears to be merely an outgrowth of the Otostapes and is therefore also of Otic origin. The Tympanic ring and extra Columella on the other hand seem to originate from the quadrate. . . ."
2. "I can see no possible connection at any time during development between this structure (i.e. Otostapes and Mediostapes) and the Hyoid Arch."
3. "Most workers have claimed a quadrate origin for the tympanic ring but only Cope and Gaupp have suggested that the extra columella might also come from the Quadrate. In the light of my own observations it seems impossible to separate the two structures in origin and both seem to me to arise from the Quadrate."

It is, of course, quite true that the frog is a highly specialised animal and its skull undergoes remarkable changes during development—but there is no reason whatsoever to believe that any phylogenetic changes could so alter the embryonic anlage as to make a vital organ like the middle ear *appear* to develop out of the chondrocranium if its original anlage had been the hyoid arch. If there is any meaning at all in the idea of homology we are entitled to say that the anuran and sauropsid middle ears are not homologous—and that fact alone suffices to discredit the orthodox theory.

On the Homology of the Urodelan Columella and the Anuran Columella

According to the functional theory the urodelan columella developed out of the hyomandibular whereas the anuran columella arose out of the chondrocranium. The ontogeny of the urodelan columella, however, is not as yet quite so definitely established and the writer hopes to elucidate that matter in a later publication. Meanwhile one piece of indirect evidence is already available from the work of Kingsbury & Reed (1904). These authors comment on the fact that in ontogeny the urodelan columella appears *before* the opercular cartilage whereas the Anuran columella appears *after* the opercular cartilage. On the basis of the Orthodox theory it is difficult to explain this reversal since it is assumed that the urodelan and anuran columellae are homologous. The ontogenetic order of appearance, however, is exactly the same as the phylogenetic order as predicated by the functional theory, that is to say, the urodelan columella represents the stegocephalian V.Sq. mechanism which preceded the operculum of the V.S. mechanism whereas the anuran columella was a late development after the V.S. mechanism had in its turn proved inadequate for the needs of the Salientia.

Degenerate or Laggard

I have already referred to the orthodox view that all but the air-sensitive ears are degenerate, and it will be clear that I do not accept that view. The auditory function is so vital to existence that it is almost impossible to envisage circumstances in which it might become unnecessary. And it is only under such circumstances that it could possibly degenerate. In the case of vision we know of animals such as the olm dwelling in complete darkness, that have become blind, but that argument does not apply to any of the animals with so-called degenerate ears. They are still assailed by meaningful sound. It is customary to speak of the middle ear of the Cetacea as degenerate. Certainly it has undergone remarkable changes as a result of reversion to an aquatic environment, especially as the terrific diving power of these animals subjects the ear to enormous pressure changes such as no terrestrial ear could possibly withstand. Nevertheless, the old idea that "whales cannot hear" seems to be quite untrue. Modern whalers certainly do not accept that statement and recent evidence rather suggests that the necessary mechanical changes have been made and that the cetacean ear is functionally quite adequate. In the case of

terrestrial vertebrates there is certainly no functional reason why they should abandon this vital organ. Smith (1938), in his account of the agamid and iguanid lizards, assumes that absence of a tympanic membrane is evidence of degeneration:

"The reason for these retrogressive changes in the ears is not evident. They are happening in creatures that live an active life on the ground and in the trees, and should benefit by having good hearing powers. It seems reasonable to assume that, in having suffered loss of the tympanum and the structures that are attached to it, they cannot hear as well as before. There is nothing in their behaviour, however, as far as can be seen, to indicate that they are handicapped by this. Whatever they have lost they seem able to get along very well without it. An orthogenetic view seems to offer the only satisfactory explanation of the phenomenon" (ibid., p. 549).

The word "orthogenesis" of course adds nothing to our understanding of the problem. There is certainly no logical reason for denying that these animals have merely lagged behind in their development. There is nothing unusual in that conception. It is indeed implicit in evolutionary theory that some species will develop a feature or characteristic more rapidly than others. Nevertheless, it must be admitted that there is something revolutionary in the suggestion that within such a restricted group as the Lacertilia there can have been such a marked diversity of development as to produce on the one hand the perfect air sensitive ear of *Gecko* and on the other hand the V.Q. mechanism of *Chamelion*—little different from the ancestral ear of the Jurassic. The logic of the argument is inescapable, however, and leads to the striking deduction that parallel evolution of the middle ear is taking place *within* the Lacertilia under our very eyes. They are all evolving air-sensitive ears—at different rates—quite independently. This conception of parallel evolution is becoming more widely accepted nowadays—one of the most striking examples being Olson's thesis (1944) that the mammals are polyphyletic. If that thesis should be acceptable it would follow that the middle ear mechanism also developed independently in the different mammalian groups. This is indeed a remarkable suggestion, but it is not without supporting evidence. Gray (1953) has recently shewn that whilst in *primates* the tendon of the tensor tympani is above the chorda tympani, in ruminants, rodents and others it is below. This can only have happened in the earliest stages of the evolution of the mammalian ear when the tensor tympani was developing out of a pterygoid muscle. Different portions of the primitive muscle must have been utilised by different groups to produce this remarkable anatomical variation.

Lastly, we must recognise one extraordinary feature about this "degeneration" theory—namely, that it envisages the ear travelling back along its own evolutionary track—since the ears to which this adjective "degenerate" is applied all represent organs which did exist in the remote past. V.Q. of modern Squamata reminds us of the pelycosaurs—V.Sq. of the modern urodeles reminds us of the Stegocephalia —V.H. of *Sphenodon* reminds us of the archosaurs. There is perhaps no reason why a degenerating organ should not *descend* its own phylogenetic tree—but it does not seem to happen elsewhere and there is no reason to postulate it in the ear when so much evidence is against it.

The Testimony of Anatomy

The Neural Relations

The relations of the middle ear structures to the facial nerve and its branches defy explanation on the orthodox theory—but present no difficulty on the functional theory.

The main difficulties are outlined by Goodrich (1930) as follows:

1. "As in Amniotes, the course of the main hyomandibular branch of the facial nerve is dorsal to the columella in Anura. Only in the Urodela does a serious difficulty arise in the comparison for here this nerve and its branches pass either ventrally to the 'stylus' and its ligaments or, as in Necturus and Proteus, it divides its jugular and alveolar (internal mandibular,

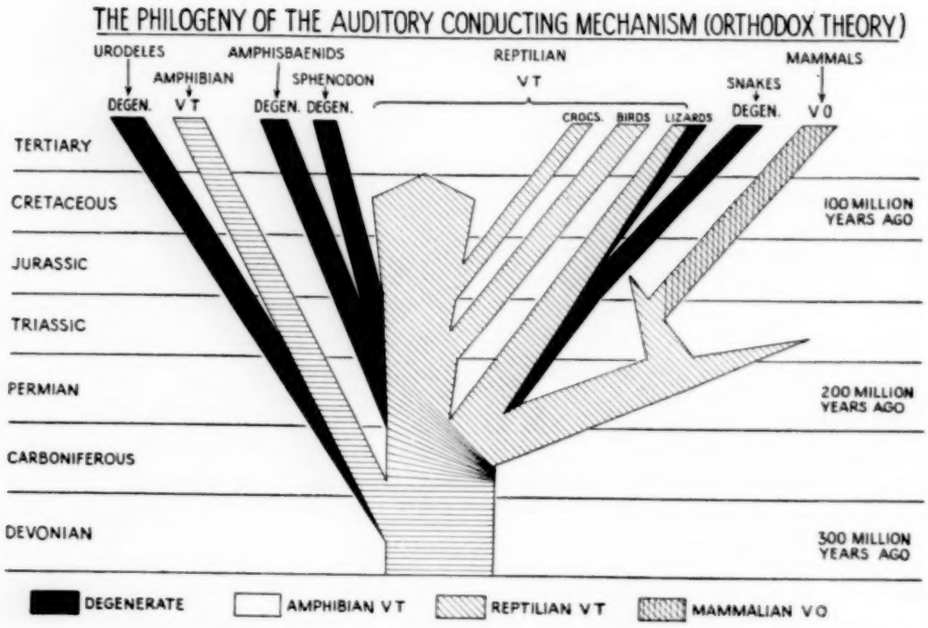

FIG. 11. Air sensitive mechanisms in amphibia, reptiles, birds and mammals are all regarded as homologous, being descended from an air-borne mechanism in the earliest amphibia. All other mechanisms, e.g. in Urodeles and many reptiles, are dismissed as degenerate.

chorda tympani) branches anterior to the 'stylus' the jugular nerve passing backwards dorsally and the alveolar anteriorly and ventrally. The ligament, then, cannot correspond to the true columella of the other Amphibia and Amniote, but may after all represent in Urodela its dorsal process and attachment.

2. "The fact already referred to that in Anura not only the hyomandibular branch of the facial nerve (jugular branch) but also the internal mandibular branch (chorda tympani) pass down in front of the tympanum, is a serious difficulty in comparing the two groups. It has already been explained that the chorda tympani in the Amniote is post-trematic but pre-tympanic, in the Anura it is both post-trematic and post-tympanic.

3. "Fully to account for the derivation of the Amniote from the Anuran condition, assuming the latter to be primitive, we should also have to suppose that the chorda tympani shifted dorsally over the end of the extra-stapedial cartilage, or that the extra-stapedial region has been developed independently in the two groups. On the other hand, it is perhaps more probable that the Anuran condition is secondary."

These reasons, amongst others, persuaded Gaupp that the anuran middle ear was not homologous with that of the Amniota. The writer has been led to precisely the same opinion as Gaupp by the functional argument and it only remains to note how easily the difficulties are overcome once the anuran amniote homology is abandoned. The above-mentioned "serious difficulty" in the Urodela due to the atypical relation of the nerve trunks to the stylus of the columella vanishes once we note that in urodeles the mechanism is V.Sq. This utilises the dorsal process of the hyomandibular and so the stylus in V.Sq. lies dorsal to the nerve trunk since the main trunk of the nerve emerges between the dorsal and ventral processes of the hyomendibular.

Goodrich indeed approximates to that viewpoint when he says:

"The ligament (i.e. of the stylus columellae), then, cannot correspond to the true columella of the other Amphibia and Amniota, but may after all represent in Urodela its dorsal process and attachment."

The paradox of the relation of the chorda tympani to the anuran middle ear also vanishes as soon as we abandon the belief that the anuran columella is homologous with that of the amniote. Once we agree that the anuran middle ear is quite independent of the sauropsid, there is no reason to expect them to mantain exactly the same neural relations. Functional requirements have determined

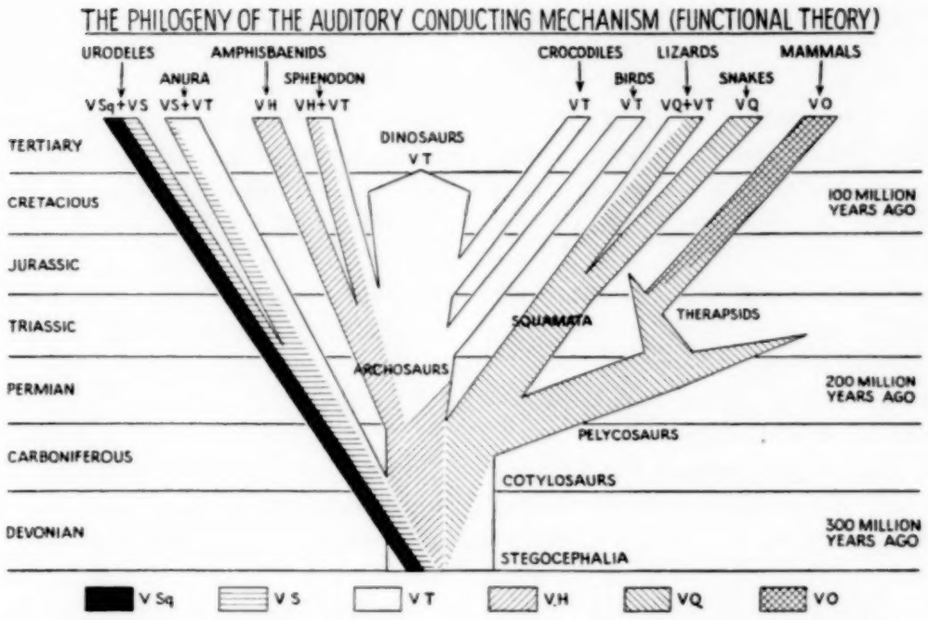

FIG. 12. The phylogeny of the auditory conducting mechanism (functional theory). The Stegocephalia started with rudimentary V.Sq., V.Q., and V.H. V.S. was also "potentially" present. Urodeles specialised in V.Sq. and later added V.S. Anura went on to add their own special type of V.T. Cotylosaurs had V.Q. + V.H. Archosaurs show the transition V.H. → VT. Squamata show the transition V.Q. → V.T. Mammalia show the transition V.Q → V.O. Thus air-sensitive hearing was a late development which emerged independently in the various groups. Modern air sensitive mechanisms are *not* homologous.

roughly similar situations but there the resemblance ends. This is analogy and not homology.

CONCLUSION

Figure 11 shows the orthodox theory with its insistence on the air-sensitive ear (V.O. and V.T.) and its dismissal of all others as degenerate. It also shows the assumption of air-sensitive hearing in the Devonian.

Figure 12 shows the functional theory and emphasises

1. The independent parallel evolution of air-sensitive hearing in Anura, Archosauria, Lacertilia and Mammalia.
2. The comparatively *late* emergence of this type of hearing.
3. The preceding bone-conducting hearing of various types.
4. The absence of degeneration.

SUMMARY

The orthodox account of the evolution of the middle ear is criticised for the following reasons:

1. It is primarily based on the anthropocentric assumption that a middle ear must include a tympanic membrane, middle ear cavity and an "air-borne" stapes.
2. It ignores all other kinds of middle ears—dismissing them as degenerate. This error follows inevitably from (1).
3. It uncritically accepts all air-borne ears as homologous ignoring the fully established fact that the sauropsid stapes arises from the hyoid arch whilst the anuran stapes arises from the chondrocranium.
4. It assumes that all these so-called homologous ears are descended from a similar ear in the common stegocephalian ancestor.
5. This leads to the intolerable paradox of the "intervening deafness" since it is clear that the pelycosaurs could not have possessed a tympanic membrane. A similar "intervening deafness" would have occurred in the therapsid-mammal metamorphosis if the orthodox account is accepted.
6. This theory indeed seems to have been based entirely on palaeontological

evidence. In each case the stapes is located and then a middle ear cavity and a tympanic membrane are created around it regardless of anatomical or other considerations. In no case has the question been asked—"How could such an ear have arisen? What purpose did it serve and how did that purpose comply with the requirements and biological characteristics of the animal?"

The writer's *functional theory* is proposed as an alternative and starts from the fact that the ear is an organ with a specific function of vital importance in the struggle for existence. It is impossible to believe that in the course of evolution it underwent almost total retrogression in animals such as the pelycosaurs which were the major group of their time. On general, physical and biological grounds we can deduce the manner in which such an organ might be expected to evolve. Such deductions are in the first place entirely theoretical but they become acceptable as a valid working hypothesis when it is found that they provide a framework in which all the available data fall harmoniously into place. The theory is essentially neo-Darwinian. Recognising the infinite plasticity of the evolutionary process, it assumes that probably every possible middle ear route has been utilised at one time or another. That leads to the deduction that at least six different middle ears have been evolved. This theoretical deduction is confirmed by the facts of morpohlogy and palaeontology. Following the neo-Darwinian line of thought it is suggested that the middle ear in any particular group at any given time must have evolved by continuous cumulative minor improvements out of the middle ear of its predecessors. The earliest middle ears were all bone-conducting mechanisms (V.Sq., V.Q., V.H.) suited to the needs of completely prostrate animals. The belief that the Stegocephalia possessed a tympanic membrane housed in the otic notch is shown to be without foundation. Subsequently, the V.S. mechanism enabled some amphibia to raise the head off the ground without the penalty of deafness. The need for an air-sensitive device did not become really acute until an erect stance was adopted. Even so, the cotylosaurs and pelycosaurs failed to evolve such an ear. It was first evolved by an obscure group of *small* reptiles that happened to be utilising V.H. This bone-conducting mechanism is also a crude air-sensitive device and enabled the early archosaurs to adopt a bipedal stance, freeing them completely from dependence on earth-borne sound. It is suggested that the triumph of the dinosaurs over the pre-mammalia was in some measure due to this fact. Detection of air-borne vibrations is shown to be much more efficient in small animals than in large. Thus the pre-mammalians failed to achieve it until the tiny therapsids evolved. The apparently purposeless, if not fantastic incorporation of the reptilian jaw joint into the mammalian middle ear is shown on functional grounds to have been entirely logical and necessary.

Modern air-sensitive ears are not homologous. The anuran stapes evolved out of the chondrocranium and has nothing to do with the others. The lacertilian, avian and mammalian middle ears all evolved independently—the only common element being the footplate of the stapes which evolved out of the ventral process of the piscine hyomandibular. It is now recognised that parallel evolution has occurred on a very considerable scale at all times, mainly in accordance with functional requirements. The evolution of the middle ear is only explicable on that basis.

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SALTATIONS IN NAUTILOID COILING

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INTRODUCTION

This paper is devoted to a critical re-examination of an old and almost a traditional concept which is usually encountered in elementary courses in paleontology. It has long been postulated that the evolution of nautiloids was characterized by a gradual and progressive change from straight shells, orthocones, to slightly curved ones, cyrtocones, loosely coiled gyrocones, and finally to nautilicones, in which the whorls are in contact or may be so closely coiled that the outer whorl embraces and may conceal the inner ones from external view. Dunbar (1924) elaborated this concept and correlated the changes in form with changes in the mode of life largely connected with varying buoyancy of the phragmocone. It was believed that the straight cephalopods held their shells horizontally in life, a matter attested by the known concentration of color bands on the dorsal side of the shell in quite a number of forms. When gas appeared in the camerae, it tended to lift the apex of the shell, and a natural response was curvature and eventually coiling. Uncoiling, in the Lituitidae, was suggested as an adaptation to an essentially planktonic existence. Asymmetrical curvature, in the shells called torticones or, later, trochoceroids, indicated a return of coiled shells to a benthonic mode of life.

There were recognized examples of both progressive coiling and of uncoiling. The ammonites supply two excellent examples of uncoiling in the Triassic Choristoceratidae and again in the Cretaceous Baculitidae. In the last ten years there has developed some controversy as to whether certain stocks represent coiling or uncoiling. The Lituitidae, long regarded as a stock which is uncoiling (Hyatt, 1900), was regarded by Schindewolf (1937, 1942) as a stock which is coiling, the coiling appearing first only in early growth stages. Controversy still surrounds the Bactritidae, alternately regarded as primitive straight Ammonoidea, secondarily straight ammonoids developed from coiled ancestors, and homeomorphs of the ammonoids developed from straight nautiloids.

These general but very interesting concepts were based upon concepts of evolution and classification of the nautiloids beyond which we have now progressed materially. Seventy-five years ago the nautiloids were classed in a few relatively simple form genera, *Orthoceras, Cyrtoceras, Gyroceras, Nautilus, Trochoceras* and *Gomphoceras.* Hyatt (1884, 1894, 1900) questioned whether this was not an oversimplification; whether there was not great homeomorphy in parallel stocks which could be differentiated by internal structures rather than external form. He developed a classification (Hyatt 1900) in which the major divisions of the nautiloids were based primarily on the form of the segments of the siphuncle, and in particular, the conformation of the septal necks. On this basis were distinguished three major and two minor orders. It was recognized that form changes were characterized by various stocks within these orders. This was an important step forward, and supplied a basis for paleontological thought which persisted for about forty years. Further investigations, however, showed that the structural types which characterized the ordinal groups of Hyatt were not monophyletic. The long septal necks characterizing the order Holochoanites appeared in only part of the

Endoceratida, which made up the nucleus of the group, and appeared in other stocks so diverse in other features, and so widely separated in time from the main stock, that homeomorphy was the only possible explanation. This was evident to Hyatt, who noted that the Cenozoic coiled *Aturia* duplicated the siphuncle wall of the true Holochoanites of the Ordovician. The order Cyrtochoanites, characterized by recurved septal necks and siphuncle segments which expanded in the camerae, showed many different origins. Teichert (1933) concluded that the actinoceroids were unrelated to other cephalopods with expanded siphuncles, and indeed, proposed that other internal structures besides the siphuncle wall supplied patterns which were a better guide to relationship than was the outline of the siphuncle alone. The ordinal groups which he proposed in general terms have much in common with those later developed independently (Flower and Kummel, 1950). It later became evident that the Cyrtochoanites of Hyatt's classification had developed many times, and that the group contained cephalopods in which remarkable convergence in this one feature had developed. It became evident that the actinoceroids, the discosorids, the oncoceroids represented three major stocks in which cyrtochoanitic structure had developed independantly, and further, that it had occurred in other minor groups, notably the Pseudorthoceratidae, the Stereoplasmoceratidae, the Paraphragmitidae of the Michelinoceratida, and in the Devonian coiled Rhadinoceratida belonging to the Barrandeoceratida, and *Germanonautilus,* belonging to the Rutoceratida.

Such a classification must collapse of its own weight. The question was with what to replace it. Flower (1950a) developed a classification of 12 orders, eventually increased to fourteen (Flower and Kummel, 1950), the two extra orders being made to include forms the origin and relationships of which were not, and still are not, clearly demonstrable. This classification, presented only in skeletal form, with brief definition of families and listing of genera, had many genera of uncertain position omitted, and indicated some matters of relationship on which there still remained much doubt. Subsequently, though almost nothing has been published, this scheme has been elaborated, the position of many of the genera then omitted being established, and many matters of phylogeny, then regarded as doubtful, having been determined. The present work is written in light of these revisions, which will be dealt with in another work now in advanced stages of preparation, together with monographic revision of certain crucial families and orders. Though not of primary importance to the present work, it should be noted that Kummel (1953) has concluded that the main group of the Nautilida stems not from smooth whorled late Paleozoic coiled shells, but from the quadrate-whorled Centroceratida. If true, the Centroceratida may be suppressed as an order, and merged with the Nautilida. However, the Liroceratidae and their descendants, the Ephippioceratidae, Paranautilidae, Clydonautilidae and Gonionautilidae, remain of uncertain origin, and may therefore be grouped properly in the order Liroceratida by themselves. The order Rutoceratida, as will be shown in a monograph of the archaic Devonian family Rutoceratidae, must be regarded as stemming not from older coiled shells as had been supposed, but from the dominantly cyrtoconic and breviconic order Oncoceratida. Probably the Rutoceratida is the ancestral stock of all Devonian or younger coiled forms, but in many cases relationships have not yet been demonstrated. The order Discosorida is now known to have developed independently of all others from the archaic Plectronoceratidae of the Ellesmeroceratida, a group recently revised (Flower, 1954). These are the only essential modifications of the phylogeny indicated in 1950.

It should be pointed out that advances in knowledge have caused us to discard some general concepts on the basis of which the old idea of progressive coiling

was based. First and perhaps most important, the orthocones are no longer regarded as primitive, but are specializations, dominantly developed in the orders Endoceratida, Actinoceratida and Michelinoceratida. It is now evident that the dominant shell form among the most ancient cephalopods, which we may safely regard as primitive, is that of a cyrtocone curved with the ventral side concave instead of convex. This is called endogastric curvature in contrast to the more common exogastric curvature, in which the ventral side is convex, the dorsal side concave.

Another important emendation involves concepts of the structure and mode of life of the orthocones. It was recognized that these animals lived holding their shells horizontally. This would plainly be impossible if the phragmocone were filled with gas, for the shell would then tend to occupy a vertical position with the apex up and the aperture down. Various hypotheses were developed to eliminate this dilemma, none very satisfactory. Some suggested that the phragmocone of these shells did not contain gas. If so, of what advantage was the phragmocone to these animals? It was hard to conceive of a successful group dragging around throughout its life a useless chambered part of the shell five to ten times the length of the living chamber. That the straight cephalopods were successful is attested by the abundance of specimens in the Paleozoic, the several hundreds of species recognized, and the large size attained by some of them. The Ordovician endoceroids attained lengths in excess of 12 feet, with only one foot of that length occupied by the living chamber; in other stocks shells of four to eight feet developed, in the Silurian, Devonian and Mississippian. That the phragmocones were of no use in the mode of life of these animals was simply unbelievable. Schmidt (1930) attempted to solve the dilemma by suggesting that the anterior camerae may have had gas, the apical camerae may have been filled with water. This would cause the lifting effect of gas to be at midlength of the animal, and not at the apical end. Of course this hypothesis is not one that can be proved or disproved from fossil material, but it is one which need no longer be considered seriously. It has been found that organic calcareous deposits of the phragmocone are confined to its apical part, that their growth is adjusted to the growth of the shell as a whole. Their function must clearly have been to shift the centers of buoyancy and gravity approximately to the mid-length of the shell. The animal was thus able to live holding the shell horizontally, while at the same time the buoyant effect of gas was such as to make the whole animal readily motile. Mode of life varies among such straight cephalopods. A few invaded plankton environments. Most forms were clearly dependent upon the bottom, but were light enough to be swimmers. A few shells with obviously excessive and heavy deposits, were dominantly bottom dwellers. These structures were largely studied by Teichert (1933) who showed estimates of their hydrostatic relationships, Flower (1939) who showed their apical and ventral concentration, and have been well expressed diagrammatically by Fischer (in Moore, Lalicker and Fischer, 1952, p. 344, fig. 9–4). Their growth, form and significance have been summarized by Flower (1955).

These two serious modifications, the concept of the endogastric cyrtocone as the archaic cephalopod shell, and the specialization of orthocones, the presence of gas in the camerae and deposits to develop a hydrostatic adjustment in the phragmocone, require serious emendations in the concept of progressive coiling. Not only is the beginning of the series different, but a reevaluation of the question of the appearance of gas in the camerae is required. In the following pages there is first a discussion of the general phylogeny of the older cephalopods with the question of mode of life and buoyancy of the phragmocone particularly emphasized, and

second, a critical survey of the examples of progressive coiling and uncoiling.

The Archaic Cephalopods

The oldest true cephalopods (fig. 1) which are adequately known are two Upper Cambrian genera of the family Plectronoceratidae. They are tiny shells, endogastric cyrtocones and orthocones, characterized in at least the young stages by short septal necks, and connecting rings outlining inflated siphuncular bulbs (Flower, 1954). They give way in the Lower Canadian to the Ellesmeroceratidae. Shells are larger, more varied in shape, but the variations still lie within the limits of endogastric cyrtocones and orthocones. Shells may be slender or rapidly expanding; apertures may be open or greatly constricted. In a species of *Burenoceras* to be described elsewhere, the specialized restricted aperture duplicates that of the Silurian *Phragmoceras,* the Devonian *Bolloceras,* and is approached by the Silurian Hemiphragmoceratidae. How these shells lived is a problem to which no really satisfactory solution has yet been found, but it is evident that the nautiloids were adapted for this mode of life, whatever it may be, four times in their evolution. Shell diversification in the Ellesmeroceratidae is an indication that members of that family were adapted to different modes of life. They are all, however, found in shallow water associations, generally in association with algal reefs, and were dominantly bottom dwellers, though many were probably capable of swimming.

There is no direct evidence of gas in the camerae of the Ellesmeroceratidae. That it was present is a hypothesis supported by its relative simplicity. This family gave rise to the Endoceratida, Actinoceratida, Michelinoceratida, and Basslerocerati da, in all of which there is reason to believe that gas was developed. The only alternative is to postulate that gas developed independently in the camerae of nautiloids at least four times, and possibly more.

Both the Plectronoceratidae and the Ellesmeroceratidae possess very closely spaced septa. When one considers the small space of the camerae, which must be still more reduced by allowing for the presence of a cameral mantle (Flower, 1954) it is doubtful whether the buoyant effect of the gas in these extremely shallow camerae would have been sufficient to more than reduce the total weight of the shell to an extremely slight degree; not enough to alter the position of the shell in a crawling and occasionally swimming animal, and not enough to require special responses to keep the shell from floating or from being held horizontally in those in which the shell was straight.

Buoyancy of the phragmocones was probably not attained simply by the sudden appearance of gas in the camerae. Rather it was probably the result of increase in the length of the phragmocone, and increase in the depth of the camerae, which increased the space available for gas, and reduced the compensating weight of the primitively closely spaced septa. It was at this period in the evolution of the nautiloids that the apices of the shells tended to point upward. For crawling shells with short phragmocones this was no handicap; when the shells became longer, they were unwieldy. Without modification such shells could not have survived long.

Orthoconic Stocks

Three great stocks of the nautiloids developed long slender shells, which were held in a horizontal position in life. These three stocks sprang from different parts of the primitive order Ellesmeroceratida. Each of them weighted down the apex of the shell by different devices. The first such stock is the order Endoceratida. The relatively large tubular siphuncles were occupied by a solid filling of endocones, which was, however, confined to the apical part of the phragmocone. Within this group straight slender shells were dominant, but the short piloceroids de-

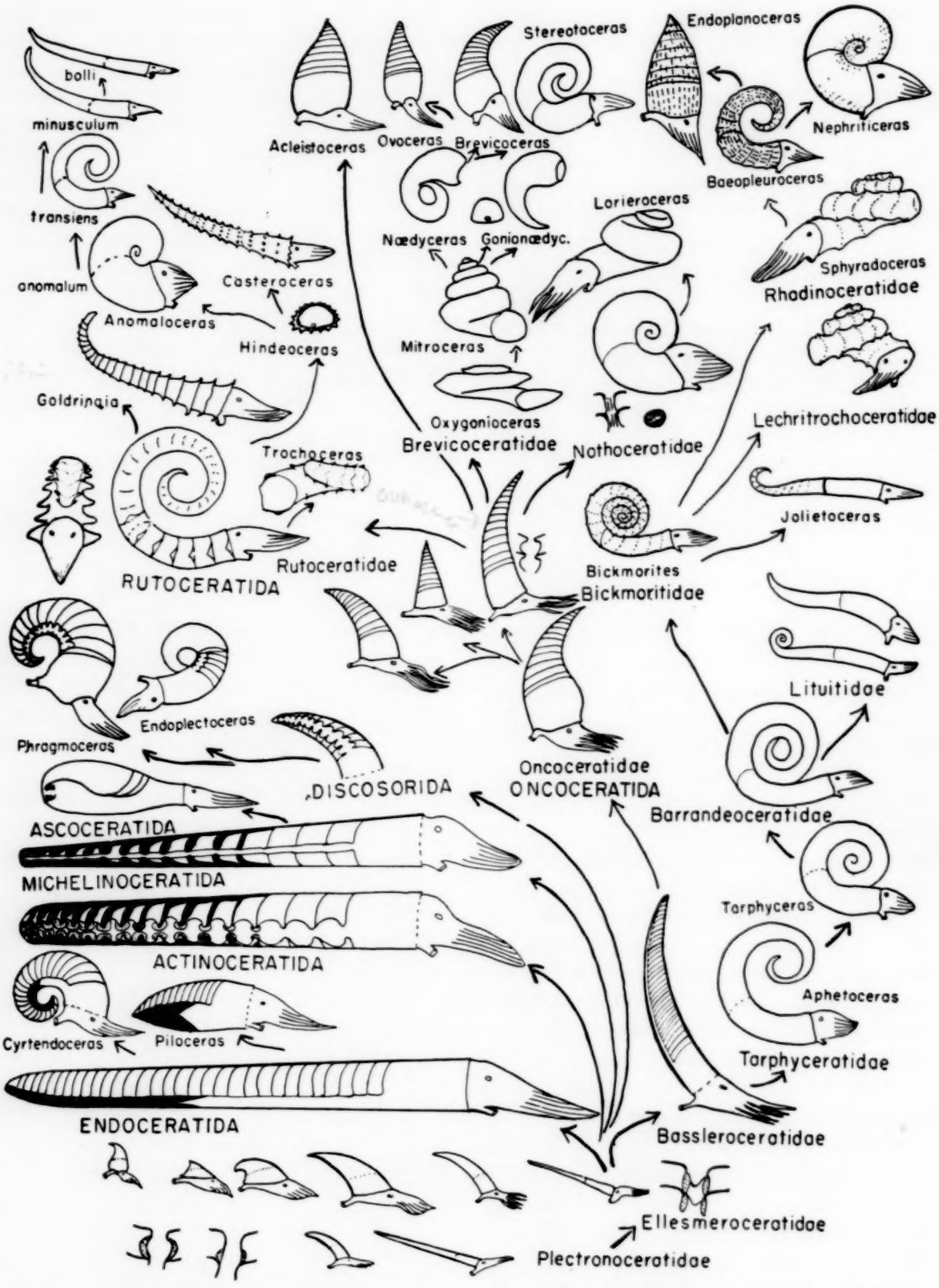

FIG. 1. Generalized diagrammatic representation of evolution of the nautiloids in the older Paleozoic, designed to show main trends of development, form types, and modifications of shell form, in particular reference to curvature and coiling. The phylogeny is necessarily selective, many families without important form changes being omitted, but most of the genera and families mentioned in the text are included and their relationships indicated. Probable appearance of the soft parts is indicated, as well as the probable position in which the shell was held in life.

veloped. There is much uncertainty concerning their mode of life. The same is true for strongly endogastric shells typified by *Cyrtendoceras,* which will be discussed later as a special case of coiling. The Endoceratida are derived directly from the Ellesmeroceratidae, the essential changes being the suppression of diaphragms and the appearance of endocones, with less fundamental changes, increase in shell size and lengthening of the phragmocone.

The second great group of orthoconic cephalopods is the Actinoceratida. There are here calcareous deposits not only in the large nummuloidal siphuncle, but also in the camerae. Teichert (1933) has shown that the mass and weight of the cameral deposits is far more significant than that of the siphonal deposits. This group is derived through the Bathmoceratidae and Eorthinoceratidae of the Ellesmeroceratida, which in turn stem from the archaic Ellesmeroceratidae (Flower, 1941).

The Michelinoceratida constitutes the third main orthoconic line. Here the siphuncle is primitively slender and small, and the weighing down of the apex is accomplished almost completely by the cameral deposits. Secondary cyrtochoanitic derivatives of this stock occur, the Stereoplasmoceratidae of the Ordovician and the Pseudorthoceratidae of the late Paleozoic. The Michelinoceratida are derived from the Ellesmeroceratidae through the Baltoceratidae, a small family of orthoconic shells differing from the Ellesmeroceratidae mainly in their broader cross section, a tendency for the siphuncle to be somewhat removed from the venter, and the suppression of the diaphragms.

Horizontal stability is accomplished in quite a different way in the remarkable Ascoceratida. First, the apical camerae moulted. Second, the mature shell developed an inflated form. Third, the camerae became prolonged over the dorsal part of the living chamber, and fused together near its base, producing a shell form unique among the cephalopods (Flower, 1941).

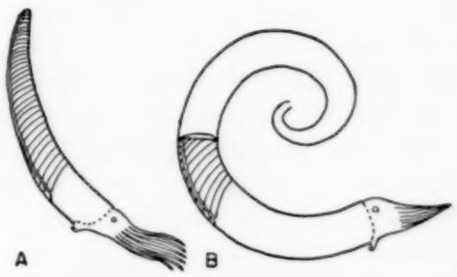

FIG. 2. Saltation in earliest known progression from cyrtocone to gyrocone. A. Basslerocerας, B. Aphetoceras. Genera are so close in cross section, surface, sutures and siphuncle that small fragments cannot be assigned to one genus to the exclusion of the other with certainty.

INITIATION OF COILING

The close of Lower Canadian (Gasconade) time marked the decline of the Ellesmeroceratidae, and the appearance of two new orders, the Endoceratida, already discussed, and the Basslerocerατida. The Basslerocerατida constitute the oldest known exogastric cyrtocones. They are larger than the ellesmeroceroids, possess longer phragmocones, but the septa are still relatively closely spaced. Such shells apparently developed exogastric curvature as a response to increased buoyancy of the phragmocone. The shells must have been held in life with the aperture pointing obliquely downward (fig. 2). It is not long after the appearance of the Basseloceratidae that the first shells appear which are gyrocones; they constitute the several species of the genus *Aphetoceras.* They appear in the late middle Canadian, and continue into Upper Canadian time. Amazingly, small fragments of shells of species of *Aphetoceras,* are extremely difficult to distinguish from portions of shells of *Basslerocerας.* The two genera agree perfectly in cross section and suture pattern. Yet there is no species known which bridges the gap from the cyrtoconic *Basleroceras,* which describes less than half a volution, to the gyroconic *Aphetoceras,* which describes at least one and a half volutions. The reason for this saltation is

apparent from figure 2. While the cyrtoconic *Basslerоceras* lived with the aperture directly obliquely downward, that of *Aphetoceras* is directed nearly horizontally, because the shell could attain balance only by having the heavy chamber beneath the buoyant phragmocone which is now coiled above it.

There remain, however, all transitions from the loosely coiled species of *Aphetoceras* to those in which the whorls are nearly in contact and more numerous, to *Pycnoceras* in which the whorls are in contact, and the higher Tarphyceratidae in which the dorsum of the outer whorls becomes concave and embraces the inner whorls to varying extents.

Among these genera there occurs a phenomenon which has been explained in the past as a gerontic reversion to an ancestral condition, a tendency for the mature part of the shell to depart from the close coil of the earlier whorls, becoming sometimes almost straight. Rather than a gerontic reversion, this phenomenon is explainable as a natural mechanical response to the development of a long and nearly tubular living chamber (fig. 3). Figure 3A shows a coiled shell with a relatively short living chamber. The living chamber is expanding gradually from its base to the aperture; consequently its center of gravity will lie anterior to its midlength, and the aperture of the shell will naturally be essentially horizontal. If, however, the rate of expansion of the shell is reduced, and the living chamber becomes long, the aperture will be directed obliquely upward as in figure 3B, producing an effect which is obviously not very advantageous to the animal for feeding or locomotion. Of such a condition among

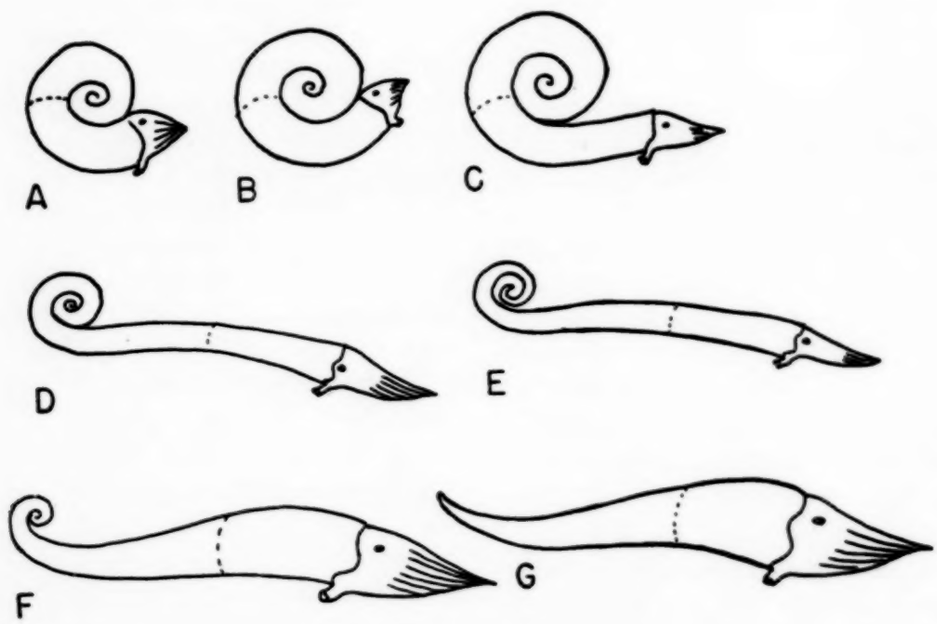

FIG. 3. Changes in shell form in early Paleozoic coiled nautiloids. A. A coiled shell with a normal living chamber which is short, expands toward the aperture. The shell would rest with the anterior third of the living chamber below, with the aperture pointing horizontally. B. A hypothetical shell in which the living chamber is lengthened, and its normal increase in diameter from base to aperture is greatly reduced. Such a shell will rest with the aperture of the living chamber pointing obliquely upward. C. Shell form common in the Tarphyceratidae, where the long slender mature living chamber shows a marked reduction in curvature, eliminating the problems encountered in B, and permitting the head-foot to contact the substratum.

D–G. Progressive stages in uncoiling in the Lituitidae, indicating the probable position of the shells in life. D. *Lituites*. E. *Angelinoceras*. F. *Rhynchorthoceras* or *Holmoceras*, two genera of very similar form, G. *Ancistroceras*. The sequence from D to G is regarded as the true direction of evolution.

the earlier coiled nautiloids, there is no evidence. Instead, the curvature of the long slender living chamber is reduced as in figure 3C, permitting the animal to maintain contact with the substratum, and producing a condition more suitable both for locomotion and feeding.

Lituitidae

In spite of marked structural differences in the wall of the siphuncle, it now appears that the Tarphyceratidae are the ancestors of a group of coiled shells which persisted throughout the Ordovician and Silurian. Most remarkable among these forms is the family Lituitidae, confined to the European Orodivician. Hyatt (1894) interpreted this remarkable family as developing first by the addition of a very long straight adoral portion to an initial portion of several whorls, so closely coiled as to develop a slight impressed zone. This is found in *Lituites* (fig. 3D). In *Angelinoceras* (fig. 3E) the initial coil is smaller and looser; the successive whorls are not in contact. In *Rhynchorthoceras* (fig. 3F) the initial coil becomes smaller and the straight portion larger and longer. In *Ancistroceras* (fig. 3G) only a slight trace of initial curvature remains, the greater part of the shell being faintly sinuate.

Schindewolf (1937, 1942) has postulated that the Lituitidae developed in exactly the reverse of the above sequence. His stratigraphic evidence rests upon the appearance of *Ancistroceras* in the upper Red Orthoceras Limestone. It rests in part also upon the conclusion that the older genus *Ancistroceras* disappears before the others, and it is the younger genera of his sequence which, while they appear essentially together, live the longest and persist into the highest stratigraphic intervals. Several reasons lead to the rejection of this scheme. First, the premise that each genus of this sequence *should* live throughout an equal span of geological time is utterly false; time and again it is the generalized type which outlives its specialized descendants. The late occurrence of *Cyclolituites* may be discounted; the genus is probably nothing more than a *Lituites* from which the adoral straight portion was missing. The only good stratigraphic evidence is the early appearance of some species of *Ancistroceras*.

However, it is one thing to postulate that the Lituitidae came from the Michelinoceratidae, and quite another to find a precise connection. The straight portions of the Lituitidae possess not only a deep hyponomic sinus, but a pair of lateral sinuses, which I am tempted to interpret as notches for the protrusion of the eyes. No known Michelinoceratida show such features. In fact, such a great morphological gap exists between the straight Lituitidae and any Michelinoceratidae that a genetic relationship between the stocks cannot be demonstrated. Acceptance of this relationship must involve a belief in connecting Michelinoceratidae which are completely unknown. On the other hand, the early coiled stages of *Lituites* and *Angelinoceras* are extremely close to *Barrandoceras* in ornament, cross section, sutures and siphuncle. For these reasons it seems evident that it is necessary to return to the concept of relationship postulated by Hyatt, because of more specific evidence in the similarity of the early stages to *Barrandeoceras*.

The uncoiled Lituitidae probably did not live as swimmers with the apex directed obliquely upward, as Dunbar (1924) suggested, but rather with the straight part of the shell directed horizontally. The buoyant effect of gas in the apical part of the shell is counteracted, at least in a large part, by deposits which developed in the camerae, which were first made known by Holm (1885) and later noted by Schindewolf (1942). The tendency may well have begun, however, as in the coiled forms noted above, in a straightening of the later part of the shell concurrent with the development of a very long and very slender living cham-

ber which is, indeed, a feature of the older Lituitidae. The stratigraphic appearance of *Ancistroceras* slightly prior to the other genera of the Lituitidae may well be fortuituous, the result of ecological or paleogeographical factors which it will probably be impossible to evaluate with certainty.

Silurian and Devonian Barrandeoceratida

Pursuing further the line of coiled cephalopods stemming from the Tarphyceratidae, one finds in the Silurian an example of uncoiling not unlike that of the Ordovician Lituitidae. The early Middle Silurian genus *Bickmorites* contains species which are coiled initially, but the mature part of the shell is straight, though in comparison to *Lituites* its coiled part is large, and the straight part relatively short. In the slightly younger genus *Jolietoceras,* the coil is reduced to an initial curvature, and the straight portion long and more expanded, very much as in *Rhynchorthoceras* and *Ancistroceras.* There the shell surface loses the costae which characterize the early stage of this genus and which also dominate the entire shell of the ancestral *Bickmorites.* It is possible that the stock developed farther, producing a generalized orthoconic shell in the Middle Silurian; if so, it has not been possible to recognize such a shell as a descendant of *Jolietoceras* among associated orthocones of the Michelinoceratidae (fig. 1).

Bickmorites is also the common point of origin of two trochoceroid stocks, the Lechritrochoceratidae of the Silurian, which are coiled sinistrally (applying the criteria of orientation as they are used in snails), and the dextrally coiled *Sphyradoceras* of the early Middle Devonian. The young stages of *Sphyradoceras* are almost identical with the adults of *Baeopleuroceras* of the late Middle Devonian. Careful examination of the similarities has led to the conclusion that *Sphyradoceras* is the only possible ancestor of *Baeopleuroceras,* and the evolutionary progression is here one of the very few examples of a demonstrable coenogenetic trend among the nautiloids. *Baeopleuroceras* itself developed a siphuncle composed of slightly expanded segments, and gave rise on one hand to the large smooth coiled shells of the Devonian, *Nephriticeras* and *Rhadinoceras,* as well as more loosely coiled lirate shells, *Lyrioceras,* and ultimately to the straight breviconic lirate *Endoplanoceras.* It is as yet uncertain whether Mississippian brevicones with relatively central siphuncles developed from this family or whether they are specialized members of the Oncoceratida.

The line of dominantly coiled cephalopods developing from the Tarphyceratidae of the Canadian cannot be traced further. While it is not impossible that the dominantly late Paleozoic Liroceratidae and their derivatives may have developed from this general stock, as yet no close connection can be found. It is necessary to turn to other stocks of the nautiloids to find the origin of the dominant groups of younger coiled cephalopods.

Oncoceratidae

From the Basslerocerati dae of the Canadian, slender exogastric cyrtocones with a ventral tubular siphuncle and thick complex connecting rings, developed the Graciloceratidae, which differ only in that the connecting ring is thin and simple. Concurrent with the appearance of the Graciloceratidae in the Chazyan, occur the first oncoceroids, which differ mainly in that the siphuncle segments become considerably expanded within the camerae, though they are still tubular in the early growth stages. The Oncoceratidae become prolific in the Ordovician, but remain within the limits of exogastric cyrtocones, some nearly straight, some with contracted apertures. *Oonoceras,* a slender exogastric cyrtocone, which appears in the Ordovician, is the stock from which an even greater expansion de-

veloped in Silurian time. Shells may be exogastric, straight or endogastric; expansion is sometimes very slight, again very rapid; apertures may be open or the living chambers may become gibbous and the aperture may be greatly contracted. The complex visored shells, the Trimeroceratidae and Hemiphragmoceratidae, are two Silurian stocks stemming from the Oncoceratidae.

In the early Middle Silurian *Oonoceras* is a slender exogastric cyrtocone, not unlike *Basslerocerας* in general form. Associated with it is the low spired trochoceroid, *Oxygonioceras*. The genera are so similar that, as in *Basslerocerας* and *Aphetoceras,* it is extremely difficult to determine to which genus fragmentary specimens should be attributed, but there are no species known which cross the boundary between cyrtocones which describe less than half a volution, and the trochoceroids which describe at least one and a half volutions in the complete shell. Clearly, there is here a second case of saltation in the degree of coiling, though this time the coil which is developed as asymmetrical.

Brevicoceratidae

Oxygonioceras is the point of origin of the Brevicoceratidae. From it developed Upper Silurian *Mitroceras,* an extremely high spired form, having, indeed, the highest spire to be found anywhere in the nautiloids. The family is unknown in the Lower Devonian. The next members to be recognized are those of the lowermost Middle Devonian, found in the Schoharie Grit of America and the equivalent horizon of Barrande Gl of Bohemia. Here are some amazing shells. *Naedyceras* is a loosely coiled slightly trochoceroid shell, the mature living chamber first slightly inflated, then slightly contracted at the aperture. Here also is *Gonionaedyceras,* a cyrtoconic edition of *Naedyceras,* but one in which the trochoceroid ancestry is strongly indicated by the askew cross section and suture pattern. Here also appear the narrow gyroceran *Gyronaedyceras,* and the broad whorled *Stereotoceras.*

Clearly, these genera are degenerate off-shoots of an ancestral stock which was a more perfect trochoceroid. Cross section, sutures and the structure of the siphuncle indicate that *Mitroceras* is this ancestor.

As the shells become shorter, less strongly curved, and the living chamber more gibbous, the breviconic genera *Brevicoceras* and *Eleusoceras* are produced. Small species of these genera exhibit rather simple siphuncles, and tend to lose the actinosiphonate deposits which have characterized older members of the stock. From such forms, including some species of *Brevicoceras,* develop a group of Devonian breviconic general including *Exocyrtoceras,* one species of which returns to the trochoceroid type of shell, and the simpler brevicones typified by *Ovoceras* and *Anglicornus.* Form deviation is rife in the family, but within the limits of trochoceroids, gyrocones and brevicones. The brevicones represent uncoiled derivatives. A radical change in the mode of life is indicated by the fact that there appear to be again no transitions from ancestral stocks of more than one whorl, and their descendants which describe less than half a whorl when complete.

Nothoceratidae

From *Oonoceras* of the Oncoceratidae, there developed in Middle Silurian time the *Nothoceratidae,* characterized by siphuncles of concave segments, filled with actinosiphonate deposits. The ancestral genus, *Blakeoceras* is a slender cyrtoconic shell, similar except in its siphuncle to *Oonoceras* which is its only logical ancestor. In Devonian time there developed from this stock a group of cyrtoconic and breviconic genera, two of which have markedly contracted apertures and are almost perfect homeomorphs of Silurian genera. *Bolloceras* is externally very similar to *Phragmoceras* of the Discoso-

rida, and *Paraconradoceras* to *Conradoceras* of the Hemiphragmoceratidae. Here also belong the breviconic exogastric cyrtocones, *Turnoceras* and *Conostichoceras*. Strong curvature and coiling is attained in three Devonian genera. *Cranoceras* Hyatt (= *Cyrtoceras, Cyrtoceratites* of authors) is a rapidly expanding strongly curved shell. No complete specimens are known, but it is evident from numerous fragments that the shells attained at least a half volution, and perhaps one complete volution. Owing to spacial problems, this genus is not included in the diagram of the phylogeny (figure 1), but two even more remarkable coiled genera are. *Nothoceras* is a stout whorled shell of several volutions and symmetrical coiling. *Lorieroceras* is a loosely coiled rather high spired trochoceroid of several volutions. Again, in the development of the coiled forms there is no good transition from the cyrtocones of less than a volution to shells of more than one complete volution, unless it is to be found in *Cranoceras,* which seems very unlikely.

The Oncoceratida underwent a great expansion in the Middle Silurian. It is possible that among the members of the Oncoceratidae, species originally described in terms of the genus *"Cyrtoceras"* there are a few other cases of the attainment of gyroconic form. Material is adequate to suggest such a development, but does not offer proof; there is always the possibility that early, very rapidly expanding stages were present. These are, however, minor developments, involving only two or three species. They are not discussed further here, and represent generic groups not as yet published.

Rutoceratidae

The Devonian marks the initiation of a new stock, the Rutoceratidae. They are dominantly gyroconic shells, the surface strikingly ornamented by frills, wings, nodes and spines, repeated at regular intervals, and plainly indicating varices of growth upon the shells. They are broad whorled, the sutures are simple, the siphuncle is ventral, and, in most forms, tubular and empty. The origin of this great group was a puzzle for a very long time; no coiled shells of earlier periods were known which resembled them at all closely. It was finally concluded that they arose, not from the older coiled shells of the Barrandeoceratida, but instead from the Oncoceratida. The evidence upon which this conclusion is based will be presented in detail in connection with a monograph of the family, now near completion. It will suffice to point out here that some oncoceroids approach the family in the breadth of the whorl, the flattening of the venter, and specialized ornament. The siphuncles of some of the Rutoceratidae are cyrtochoanitic, sometimes they contain actinosiphonate deposits. For some years the possibility was considered that the Devonian Rutoceratidae, as here defined, was actually comprised of two unrelated stocks exhibiting a remarkable contemporaneous convergence of form and structure. This concept had to be abandoned because (1) of complete intergradation among the two groups of Devonian species and genera and (2) of complete failure to trace the orthochoanitic elements of the stock to any even remotely likely ancestors in the older strata. It came, instead, to be recognized that the expanded siphuncles and actinosiphonate deposits were relic features, retained from their ancestors in the Oncoceratida. This hypothesis was strengthened greatly when it was realized that these features were almost completely confined to the older genera, *Trochoceras, Muiroceras, Rutoceras,* and *Adelphoceras.*

This family, from its appearance in the Lower Devonian, is composed dominantly of gyroconic shells. Some develop closer coiling and impressed zones; in some genera there are all transitions between the gyroconic shells and those which are more closely coiled, as was found in the Tarphyceratida. There are some trochoceroid shells which depart from the nor-

mal symmetrical coil, but the departure is generally extremely slight. Sometimes it is only evident that the inner whorls are slightly askew from the plane of symmetry of the outer whorls. Again, as in *Trochoceras* and one genus as yet undescribed, the departure is marked, but the spire is never as strongly elevated as in the Lechritrochoceratidae or in *Sphyradoceras* of the Rhadinoceratidae.

Within the Rutoceratidae are several remarkable cases of uncoiling of the shells. The first is found in the frilled shells, dominantly of the American Devonian, of the genus *Golringia*. Most species of this genus are gyrocones, but one group, typified by *Goldringia eugenia* (Hall) of the Schoharie grit, is a shell which is cyrtoconic in the young and essentially straight in the adult portion. Unlike the Lituitidae, the phragmocones are long, the living chambers short, and no cameral deposits are developed. So close is the relationship with the true gyrocones of similar structure, that separation of the two groups into separate genera is not feasible. Relationship is close, there is here one of the very few good cases of transition from a gyroconic to a cyrtoconic shell. Did the cyrtoconic and gyroconic shells live similarly? It seems highly probable. If so, the cyrtoconic shells could have lived with the living chamber in a horizontal position only if the gas in the camerae was reduced.

In the upper half of the Middle Devonian of Bohemia are two very closely related genera, *Hercoceras* and *Anomaloceras*. *Hercoceras* is closely connected to the main stock of the Rutoceratidae. Typically, it is a gyroconic shell with a pair of large lateral spines, but in some species the spines become reduced and the shells may eventually become essentially smooth. *Anomaloceras* springs from such species, and differs from them first by the fact that though the coil of the shell is symmetrical, the siphuncle is moved definitely to one side of the center. This is true of the type species, *Anomaloceras anomalum* (Barrande). Existing in the same beds are other shells which agree with this one in cross section, sutures, and the askew siphuncle, but differ in showing all variations in coiling (see fig. 1). One, Barrande described as *Trochoceras transiens*, though it is properly a gyrocone. Another he called *Gyroceras minusculum*, a loosely coiled shell which describes little more than a complete volution. A cyrtoconic shell he named *Cyrtoceras bolli*. These species are connected by transitional forms, not yet illustrated or described. They are evidently more closely related to each other than to any known species. They are therefore all being referred to the genus *Anomaloceras*. This procedure will come as something of a shock to paleontologists, among whom the degree of coiling of the shell has been almost traditionally a generic criterion. However, it is felt that the erection of a separate genus for each of these species is unjustified; the names would serve no good purpose; they would tend to obscure rather than to show relationship. Undescribed forms separating these species present the interesting problem of where good boundaries could be drawn between such possible generic groups. Here is plainly one of the most remarkable and most perfectly documented cases of gradual uncoiling, among the nautiloids.

A third case of uncoiling occurs in the same family. The genus *Hindeoceras*, a gyrocone with circles of spines and nodes developed around the circumference of the shell at regular intervals, is plainly the origin of *Casteroceras*, which differs from it mainly in that the shells are cytoconic, though only faintly so, and sometimes essentially straight. As yet no good transition has been found between these genera, though in the monographic study of the family 40 species of *Hindeoceras* and 5 species of *Casteroceras* are recognized. Uncoiling must again be invoked to include the genus *Tylorthoceras*

in the family, but this genus is not as yet closely connected with the others. Even its precise position and origin among the other genera remains doubtful.

In the Rutoceratidae uncoiling occurs in at least four distinct lines of descent; in two of them it is clearly a gradational process. With such gradual transitions, it appears impossible that any such radical change in the mode of life could have occurred, as evidently did between the primitive cyrtocones and gyrocones. The only possible explanation is that the cameral gas must have been reduced so that the cyrtoconic derivatives could have held their living chambers in an essentially horizontal position.

It should be noted that in figure 1 *Hercoceras* is not represented owing to problems of space. *Anomaloceras* is there indicated as stemming from *Hindeoceras*. *Hindeoceras* is actually the ancestor of *Hercoceras* as will be shown more fully in the monograph of the Rutoceratidae now near completion.

Later Coiled Stocks

Kummel (1953) has confirmed the suspicions of the writer (Flower 1952) that the great group of late Mesozoic and Coenozoic coiled shells included in the Nautilida (Flower and Kummel, 1950) springs not from the smooth involute shells of the Late Paleozoic, but from the shells of quadrate whorl formerly included in the Centroceratida. Evidence of the origin of the Nautilida (as now expanded to include the Centroceratida of Flower and Kummel, 1950) is dubious, (Flower, 1952), but the scant evidence seems to favor an origin of that group in the Rutoceratidae rather than in any other stock. Probably the small Late Paleozoic order the Solenochilida has its origin also in the Rutoceratida, though again we must await more conclusive evidence of a closer connection before this can be considered as established. Neither the Solenochilida nor the Nautilida can be at all closely connected with any other stock. On the other hand, the late Paleozoic Liroceratidae and their derivatives in the Mesozoic, cannot be traced back to any known ancestor in Devonian or older strata.

The interesting fact is that the dominant stocks of coiled nautiloids from the Late Paleozoic to the recent, appear to stem, not from the Tarphyceratida and Barrandeoceratida, the coiled shells of the Ordovician and Silurian, but from stocks which, at the time these coiled shells flourished, were the cyrtocones and brevicones of the Oncoceratida.

Within these later stocks there is no recognized transition from the cyrtocone to gyroconic shells, though there are all variations from gyrocones to evolute and strongly involute nautilicones. Cases of uncoiling are probably confined to the family Solenochilidae, though possibly others may be established in the essentially European Mississippian family Triboloceratidae.

Endogastric Coiling

Endogastric coiling (shells coiled with the ventral side concave instead of convex) is rare among the nautiloids. As the shell is curved and coiled so that the apex is directed first backward, and then down, the generalizations relevant to exogastric coiling cannot possibly apply. There is much uncertainty as to how the endogastrically coiled cephalopods lived, and no convincing ecological reason why such shells should have developed.

The first case so far described in the stratigraphic column is the coiled *Cyrtendoceras* of the upper grey Orthoceras limestone of Sweden. A similar but more rapidly expanding form from the Middle Canadian El Paso limestone now awaits description. Beyond the fact that these shells developed within the Endoceratida, there is little certainty as to their exact origin; it is believed that the El Paso genus is derived from the family Piloceratidae, the younger *Cyrtendoceras* from the essentially straight and more

specialized Endoceratidae. It is not evident in what position the shell was held in life. The strong hyponomic sinus does suggest that the animal was probably capable of swimming.

The only other cases so far known occur in the anomalous order Discosorida. This is a group of uncertain origin, but probably developed from the Ellesmeroceratida independent of other cyrtoconic stocks. The older Ordovician forms are exogastric cyrtocones, but straight and endogastric forms develop in the later Ordovician and Silurian. Only one line of descent is of interest to the present study, that starting with the Cyrtogomphoceratidae, large endogastric brevicones of the Upper Ordovician, from which stem the Phragmoceratidae of the Middle Silurian. In this stock developed an endogastric trochoceroid genus, *Endoplectoceras*, confined to the Middle Silurian of Bohemia. The breviconic *Phragmoceras*, with its amazingly contracted aperture, consists largely of cyrtoconic species, but there are a few, largely known again from the Middle Silurian of Bohemia, in which the shell is so strongly curved as to describe slightly more than a single volution. No convincing interpretation of the mode of life of *Phragmoceras* has ever been presented. It may have been a shell which floated with the aperture directed downward, obtaining what food it could by extending trailing tentacles from the restricted aperture; it is equally possible that the entire foot might be protruded through the aperture, and that the animal was dominantly a crawler, not unlike the living gastropod *Cypraea*. The strong hyponome suggests that it was a swimmer; its association in shallow water deposits, often in conjunction with coral reefs suggests the opposite interpretation. One fact is evident, however. In both *Phragmoceras* and *Cyrtendoceras* whatever weight the siphuncle adds to the phragmocone is so placed that it lies as close as possible to the center of the coiled shell.

Conclusions

The development of gas in the camerae of nautiloids is an early, and very possibly an archaic feature, beginning certainly in the Ellesmeroceratidae, and very possibly in the Plectronoceratidae which contains the oldest known nautiloids (Flower, 1954). Primitive cephalopods were dominantly endogastric cyrtocones, probably dominantly crawlers. The short phragmocones and closely spaced septa left little space for gas, and were probably not significantly buoyant.

When their descendants developed longer phragmocones with deeper camerae, and generally larger shells, the buoyant effect of cameral gas became proportionately greater than the weight of the shell parts, and thus came to have a definite lifting effect upon the apical part of the shell. Response to this phenomenon was varied; three major groups of straight shells weighted the apical part of the shell in three different ways (fig. 1) by accessory shell parts. In the Ascoceratida the byoyancy of the tip was reduced by natural truncation. Other forms responded by curving exogastrically. This is first encountered in the Basseroceratidae of the Middle Canadian, a family which is the ancestor of the main groups of exogastric curved and coiled shells.

The change from a cyrtoconic shell of less than half a volution to a coiled one comprising at least one and a quarter volutions can be demonstrated at four points in nautiloid evolution: (1) development of *Aphetoceras* from *Basslerоceras* in the Canadian, (2) development of *Oxygonioceras* from *Oonoceras* in the Middle Silurian, (3) development of the coiled genera *Nothoceras* and *Lorieroceras* in the Devonian Nothoceratidae, (4) development of the oldest Rutoeratidae (*Ptenoceras* and *Trochoceras* of the Lower Devonian) from Silurian Oncoceratidae. In every case the change is abrupt, though in two of the series, that of *Basslerоceras* to *Aphetoceras* and that

of *Oonoceras* to *Oxygonioceras,* there is abundant evidence of an intimate relationship between the cyrtoconic ancestors and their coiled descendants. So closely similar are the species, that the absence of transitional forms cannot be convincingly attributed to incompleteness of the geological record. The change from the cyrtocone to the gyrocone or torticone is saltational, never gradational. One may of course only speculate as to the genetic factors involved. Doubtless increase in coiling is to be attributed to genetic causes. It is possible to conceive of the saltation as an example of quantum evolution, an all-or-none reaction. However, one may wonder whether the location of this saltation at the same point in form changes which are otherwise gradual and progressive, is not ecologically controlled. It seems eminently improbable that the same genetic factors should develop four times in nautiloid evolution, always being made manifest at the same point in coiling, jumping from one side to another of a morphological gap. It seems the more unconvincing in view of the fact that this gap is one at which the change in form requires a major modification in the mode of life. The suddenness of this change is significant because, from the point of view of shell form and structure, it is not required; indeed, it was previously unsuspected. It is worthy of note because sudden changes of this sort are the exception in nautiloid evolution. There are, to be sure, some comparable saltations, but they involve the appearance or disappearance of structures, or else processes which by their very nature must be all-or-none reactions. The best example is the appearance of truncation in the ascoceroids (Flower, 1941). Either the shell apex was retained or it was lost. They could not, as suggested to the Lord High Executioner of the Mikado, cut it half off. Others are the appearance of annulosiphonate deposits in siphuncles where before there were no such structures, the appearance of endocones, the development of the mantle over the shell in the first coleoids and the secretion at once of a very extensive rostrum. Other seeming examples are more apparent than real, but a discussion of them is hardly pertinent at this time. It should however be noted that the appearance of the remarkable vascular system of the actinoceroids and the appearance of actinosiphonate deposits in the Oncoceratida are both sudden only because excessive growth of calcareous deposits in each case conforms to structures of the soft parts which were developed long before.

While there is no good example in the nautiloids of gradual progress from the curved to the coiled shell, uncoiling seems to be generally a gradual process. Among older nautilicones are many forms in which curvature of the mature living chamber is markedly decreased. Early writers regarded this as a gerontic return to an ancestral condition. Instead, it appears to be a simple mechanical response to the development of a very long and slender living chamber, enabling the aperture to point horizontally instead of obliquely upward. Thus the protruded cephalopodium could maintain contact with the substratum. In the Lituitidae uncoiling beyond this point is gradual, and the end result is a group of shells which are essentially straight. Deposits developed in the camerae, permitting the living chamber, now excessively long, to be held horizontally. A parallel development, but one which apparently died out before perfectly straight shells were developed, is found in the progression from *Bickmorites* to *Jolietoceras* in the Silurian. No cameral deposits are known, and the gradual nature of the change is less clearly documented, probably because of the small number of species known in this series.

In the Rutoceratidae gradual uncoiling is well substantiated by the numerous species of *Goldringia,* and again in *Anomaloceras.* No transitions between gyroconic ancestors and the nearly straight *Casteroceras* and *Tylorthoceras* are

known, but again, the species concerned in these series are relatively few, and our evidence here is probably yet very incomplete.

It is highly unlikely that in these cases of uncoiling any change in the position of the living chamber occurred. Special modifications were required to counteract the lifting effect of gas in the apical part of the shell. In the Lituitidae alone cameral deposits supply an explanation. It is highly probable that in the other uncoiled stocks the gas in the camerae was simply reduced.

On the other hand, abrupt changes in uncoiling appear to exist where the end result was a shell in which the aperture must have pointed obliquely or sometimes directly downward instead of horizontally. The end result is here not a slender orthoconic shell, but a breviconic cyrtocone, often with a contracted aperture. The evidence of color bands, admittedly fragmentary though it is, is unanimous in indicating that these shells were held in life with the apex pointed upward, the aperture downward. It therefore appears that where uncoiling does involve a radical change in the mode of life, it may be saltational.

Endogastric cyrtocones were developed (1) in the archaic nautiloids, (2) in specialized Endoceratida, (3) in specialized Oncoceratida, (4) in specialized Discosorida. With the possible exception of the endoceroids, concerning the mode of life of which almost nothing is known, these were stocks which held their shells with the apex pointing upward. Endogastric shells which attain a real though a loose coil of more than one volution are exceptional. In the Endoceratida there is one undescribed genus and *Cyrtendoceras*. In the discosorida there is the small genus *Endoplectoceras*, and coiling is attained in a few species of *Phragmoceras*. In *Phragmoceras* the development is gradual; in the other forms it does not appear to be, but the record is too fragmentary to be conclusive. In any case, the physical and mechanical considerations involved in exogastric coiling cannot possibly be relevant.

Once a shell of more than one complete volution is attained, there may be all gradations from those with loose free whorls, to those with whorls in contact, and to those with the outer whorl embracing and concealing the inner whorls. There appears also to be a close relationship between symmetrically coiled shells and those in which the spire is slightly askew. Such gradation in this respect occurs within the Rutoceratidae, that generic distinctions on the basis of slight asymmetry of the coil is impossible. Again in the Brevicoceratidae, a very close relationship appears to exist between the gyroceran forms and the torticonic genera. On the other hand, no close connection is shown between the gyroconic Bickmorites and the torticonic descendants of the Lechritrochoceratidae. There is no known gradation of Sphyradoceras to the ancestral Bickmorites or its descendant Baeopleuroceras. The oldest torticone, *Aethoceras* Teichert and Glenister (1954) is not intimately connected with the symmetrically coiled and closely related other genera comprising the Tarphyceratidae. The evidence suggests that most coiled nautiloids were essentially bottom-dwellers, capable of swimming but without much ability to direct their course in the water. Assuredly, there is no wide gap between such forms and the trochoceroids, long regarded as a benthonic adaptation, though the spire of the torticone would make swimming difficult or perhaps impossible without special adaptations of the soft parts, a matter on which we have no basis for conjecture.

There is clear evidence of a saltation in an otherwise gradational series in the progress from a curved to a coiled shell in evolutionary series showing increased curvature. There is evidence of a similar jump in uncoiling only where the end result is a breviconic cyrtocone. That

these morphological gaps coincide with points in evolution at which a profound change of the life position of the shell and aperture occur, is hardly to be dismissed as a coincidence. It would appear therefore that the essential factors controlling stabilization beyond the saltation are ecological and not genetic.

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ON INTERRELATIONSHIPS OF THE CELLULAR ANTIGENS OF SEVERAL SPECIES OF STREPTOPELIA[1]

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INTRODUCTION

It has been shown that the antigens of the red blood corpuscles, which distinguish one of a pair of species of doves or pigeons from the other, segregate in the backcross progeny according to genetic expectation (for references, see Irwin, 1951). These findings, coupled with those on the heredity of cellular antigens within various species (for references, see Race and Sanger, 1950; Rivière and Eyquem, 1953; and Wiener, 1943), indicate strongly that all the antigens of the blood cells are gene-determined. Hence the interrelationships of the cellular antigens of various species would be an index of the genetic relationships of these species, even though the cellular antigens might not be identified separately. The interrelationships of the cellular antigens among eight species of *Streptopelia* are presented in this report, from which parallel genetic interrelationships may be inferred. The sharing or non-sharing by other species of pigeons and doves (Columbidae) of the cellular antigens which distinguish Pearlneck (*St. chinensis*) from Ring dove (*St. risoria*) have been presented in a previous paper (Irwin, 1953), illustrating that a cellular substance which distinguishes one species from another, as Pearlneck from Ring dove, may be shared in whole or in part by one or more other species. The interrelationships to be described in the present report will necessarily be based in part upon those antigenic substances by which Pearlneck is differentiated from Ring dove, and in part upon other substances.

MATERIALS AND METHODS

In the present investigation comparisons have been made of the interrelationships of the cellular antigens (also called cellular characters, antigenic characters, cellular components or antigenic components) among representatives of eight species of the genus *Streptopelia: capicola, dussumieri* or *bitorquata, humilis* or *tranquebarica, orientalis, chinensis* or Pearlneck, *risoria* or Ring dove, *semitorquata*, and *senegalensis* or Senegal. Representatives of these species were purchased from various dealers and fanciers in this country. These species are indigenous to various areas in the Old World. Thus, *capicola, semitorquata* and *senegalensis* or Senegal are native to Africa, *dussumieri* to the general area of the Philippine Islands, *chinensis* or Pearlneck to southern China and contiguous areas, *humilis* (*tranquebarica*) to northern India and eastward to the Philippine Islands, and *orientalis* to northern China and areas to the west. The wild species from which *risoria*, the domesticated Ring dove or Ring neck dove, is descended is not known with certainty, although it is probably either *roseogrisea* or *decaocto* (these may not represent different spe-

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cies), with a range from India as far westward as the Anglo-Egyptian Sudan.

The comparisons of the cellular antigenic characters of these eight species have been made on the basis of the presence or absence of agglutination of the red blood corpuscles of the various species of Streptopelia by specially prepared "test-fluids" or "reagents," as will be described. The antisera from which these reagents were prepared were obtained by injection of rabbits with washed erythrocytes from representatives of each of the species. The details of these methods, and of the agglutination tests, have been described in previous papers (Irwin and Cole, 1936; Irwin, 1939).

Explanatory Remarks

An antiserum produced by the injection of the red blood corpuscles of a species of *Streptopelia* contains antibodies which cause the agglutination, at high dilutions of the antiserum, of the blood cells of the species used in immunization. The highest dilution of these antisera at which agglutination was visible was usually one part of antiserum to 15,000 or 30,000 parts of saline, isotonic for bird cells. The antiserum against the cells of any of these species also agglutinates the cells of the other seven species, usually at the same dilution that obtained for the immunizing cells. Hence, no differentiation by such a test was possible among the cells of the various species. Further, on the assumption that the blood cells of each species have numerous discrete antigenic substances, these results indicate that each of these species shares an appreciable proportion, probably the greater proportion, of its antigenic substances with the other species, as identical or similar cellular components. On the assumption that these antigenic substances of the blood cells are gene determined, it follows that the substances which are *common* to any two or more species are indicative of the genes with antigenic effects which are identical, or at least similar, in the species.

The technics of antibody absorption allow a very precise means of differentiating the cells of each species from those of another species. For example, an antiserum produced against the cells of Pearlneck, when absorbed with the cells of another species, as Ring dove, will agglutinate the cells of Pearlneck at high dilutions (1:15,000 or higher) but will not react with those of Ring dove at the dilution of antiserum used in the absorption, usually 1:60 (Irwin and Cole, 1936). The antibodies reactive with the antigenic substances *shared* by the two species have been removed from the antiserum by the above process, and only those *specific* to Pearlneck, in comparison with Ring dove, are left in the antiserum. The antiserum to Pearlneck has thus become a "reagent" or "test-fluid" which will react strongly with Pearlneck cells but not at all with those of the contrasting species, Ring dove. These antigenic characters, then, represent a qualitative difference of Pearlneck cells as contrasted to those of Ring dove.

An antiserum prepared against the blood cells of a species may be pictured as being complementary to the various antigens of the cells used in the immunization, in so far as each antigenic substance has induced the formation of its specific antibody. In other words, the phenotype of the species has in effect been transferred to the immune serum and, by the process of antibody absorption, various fractions of the antibodies (complementary to the phenotype of the cells used in the absorptions) can be removed from the antiserum at will. Thus, if the antiserum to Pearlneck cells, following absorption with Ring dove cells, is reactive with the blood cells of other species, it means that these other species share with Pearlneck one or more identical or similar antigenic substances—but not necessarily the same similar substances—by which Pearlneck is differentiated from Ring dove. This method of comparison of the interrelationships among the various species of these tests is a means of analyzing

some of the qualitative differences among species.

Further, the segregation of these specific antigenic substances of Pearlneck in backcrosses to Ring dove of species hybrids between Pearlneck and Ring dove (Irwin and Cole, 1936) has given evidence that these differentiating characters are hereditary. Actually, the specific components of Pearlneck have been separated into at least nine independently inherited substances, as a result of backcrossing selected backcross hybrids to Ring dove (Irwin, 1939). From these results, and others which substantiate them completely (Irwin, 1951) it appears reasonable to conclude that the differentiating antigenic substances of the blood cells of all the species on which tests have been made, and presumably of all other species, are gene controlled. In short, the inference seems inescapable that all the cellular antigens of any species are the products of genes.

The various readings of the agglutinations of the cells of the eight species of Streptopelia that are given in table 1 represent the results obtained usually of two or more separate tests, performed at different times. Occasionally, at different times of testing, the same combination of a reagent and cells produced different results in that no agglutination may have occurred at one test as compared with definite clumping at another. In such cases, both reactions are given, unless the reciprocal test (see below) has made the agglutination the more probable, and in such cases only the positive reaction is recorded in the table.

Experimental Results

As stated above, the antiserum to each of the eight species tested was reactive with the cells of the other species at the same, or nearly the same, dilution of the antiserum that obtained for the immunizing cells. However, as is given in table 1, the specificities of the reactions of antisera to each species following the absorptions with the cells of representatives of the other species revealed a variety of relationships. Although the results of the tests are not given in the table, the absorption of each antiserum by the cells of the species used in immunization resulted in the removal of all the antibodies from the antiserum. This result would be expected unless (a) there were individual differences in cellular antigens among the representatives of the species furnishing the immunizing cells, and these were expressed at the relatively high dilutions of antisera which were used in these tests, or (b) unless the corpuscles of a species engendered antibodies during immunization to antigenic substances not contained by, or recognizable in, the immunizing cells. (Authentic cases of this latter kind are extremely rare, if any exist.) Another possibility would be that the injected animal *prior to immunization* possessed antibodies which showed a differential reactivity towards the cells of different species. Normally occurring antibodies in rabbit serum to the cells of these species of doves have not been observed at the dilutions of serum used in these tests, and probably have not been a complicating factor.

Analysis of the Antiserum to *capicola*

It may be noted in table 1 that, in general, there were about as many specificities in reactivities of the various reagents for each antiserum as there were different species whose blood cells were used in the absorptions. The accuracy of the observations may be judged by the agreement of the reciprocal reactions of the agglutinations throughout the table. For example, anti-*capicola* serum absorbed by the cells of *dussumieri* agglutinated the cells of *humilis* definitely but weakly (column 5, line 2). Hence, the antiserum to *humilis*, when absorbed by *dussumieri* cells, should have reacted with the cells of *capicola* and this reaction was observed (column 3, line 17).

There are, however, some of the agglutination reactions in which there was not agreement in the reciprocal tests. For example, the antiserum to *capicola,* when exhausted with *humilis* cells, agglutinated those of *dussumieri* (column 4, line 3) but the antiserum to *dussumieri* when similarly absorbed with *humilis* cells did not react with the cells of *capicola* (column 3, line 10). Each such reaction in table 1 which represents a lack of agreement in reciprocal reactivity is assigned a common superscript—1 to 18, inclusive. Various explanations could be offered for the eighteen exceptions to complete agreement in the reciprocal reactivities of table

TABLE 1. *Agglutinations of the erythrocytes of eight species of Streptopelia with reagents prepared from the respective antisera to each species*

Antiserum to	Absorbing cells	Test cells: *capicola*	*dussumieri*	*humilis*	*orientalis*	Pearl-neck	Ring-dove	*semitorquata*	Senegal	Line
										1
	dussumieri	C	*2*	±	*2*	+	±	+	++	2
	humilis	C	±[1]	0	±	+	±	+	++	3
	orientalis	++	0	0[2]	0	±	±	+	+	4
capicola	Pearlneck	C	C	C	C	*2*	C	C	C	5
	Ring dove	C	++	++	++	+	0	±	++	6
	semitorquata	C	++	++	++	++	±	*2?*	C	7
	Senegal	C	C	C	C	±	C	C	*2*	8
	capicola	*2*	C	++	++	+	*2*	*2*	+	9
	humilis	*2*[1]	++	*2?*	±	0[3]	?	0	0[4]	10
	orientalis	*2*	++	±	0	?	±	±	±	11
dussumieri	Pearlneck	C	C	C	C	0	C	C	C	12
	Ring dove	++	C	C	C	C	0	0, ?	++	13
	semitorquata	++	C	C	C	C	0, ?	0, ?	C	14
	Senegal	C	C	C	C	*2*[5]	C	C	0	15
	capicola	*2*	++	++	++	++	±[6]	*2*	++	16
	dussumieri	+	*2*	++	±	±	*2*	±[7]	*2*[8]	17
	orientalis	+[2]	++	++	*2*	*2*[9]	*2*	±	±	18
humilis	Pearlneck	C	C	C	C	0	C	C	C	19
	Ring dove	++	C	C	C	C	0	±[10]	C	20
	semitorquata	++	++	C	++	++	0, ?	0	++	21
	Senegal	C	C	C	C	±	C	C	0	22
	capicola	*2*	++	++	C	++	*2*	*2*	++	23
	dussumieri	*2*	0	±	++	++	*2*	*2*	±	24
	humilis	±	++	*2*	C	*2*[11]	*2*	*2*	*2*[12]	25
orientalis	Pearlneck	++	++	++	C	0	C	C	++	26
	Ring dove	++	C	C	C	++	0	±[13]	++	27
	semitorquata	++	C	C	C	++	0, ?	0	C	28
	Senegal	C	C	C	C	0[14]	C	C	0	29
	capicola	*2*	++	++	++	C	±[15]	±[16]	++	30
	dussumieri	++	*2*	±	+	C	±	±	++	31
	humilis	++	++[3]	*2*	+[11]	C	±	±	++	32
Pearlneck	*orientalis*	++	++	±[9]	*2, ?*	C	±	±	++	33
	Ring dove	++	++	++	++	C	0	±	C	34
	semitorquata	++	C	++	C	C	±	0	C	35
	Senegal	++	++[5]	++	++[14]	C	0, ?	±[17]	0	36
Column 1	2	3	4	5	6	7	8	9	10	

Symbols: C = complete agglutination, ++ = strong agglutination, + = definite agglutination, ± = definite but weak agglutination, ? = questionable agglutination, 0 = no agglutination—at the first dilution (usually 1 : 90) of the reagent-cell mixture; *2* = no agglutination at the second (doubled) dilution of the mixture.

TABLE 1—*Continued*

Antiserum to	Absorbing cells	Test cells: *capicola*	*dussumieri*	*humilis*	*orientalis*	Pearl-neck	Ring-dove	*semitorquata*	Senegal	Line
		1	2	3	4	5	6	7	8	1
	capicola	0	0	0^{6}	0, ?	0^{15}	++	±	0	37
	dussumieri	++	0	±	0	±	C	C	+	38
	humilis	++	0	0	0	±	C	C	+	39
Ring dove	*orientalis*	++	±	±	0	+	C	++	+	40
	Pearlneck	C	C	C	C	0	C	C	++	41
	semitorquata	+	±	±	0, ±	±	++	0	±	42
	Senegal	C	C	C	C	0	C	C	0	43
	capicola	?	*2*	*2*	*2*	2^{16}	?	++	±	44
	dussumieri	±	*2*	2^{7}	*2*	±	C	C	±	45
	humilis	+	?	*2*	*2*	?	C	C	±	46
semitorquata	*orientalis*	±	?	±	*2*	?	C	C	±	47
	Pearlneck	++	++	++	++	*2*	++	C	±	48
	Ring dove	±	±	0^{10}	0^{13}	±	0	+	0^{18}	49
	Senegal	+	+	+	C	2^{17}	C	C	*2*	50
	capicola	?	++	++	++	++	?	±	C	51
	dussumieri	++	0, ?	$++^{8}$	++	++	+	+	C	52
	humilis	++	$++^{4}$	0	$\pm^{12}$	++	±	++	C	53
Senegal	*orientalis*	++	+	±	?	C	+	++	C	54
	Pearlneck	++	++	++	++	0	±	±	C	55
	Ring dove	++	C	C	C	C	0	$\pm^{19}$	C	56
	semitorquata	++	C	C	C	C	0, ±	0, ?	C	57
Column 1	2	3	4	5	6	7	8	9	10	

1, and those most probable appear to be (a) the individual response of the rabbits to immunization of which examples have been reported elsewhere (Irwin and Golden, 1942), and (b) differences in sensitivity of the blood cells in the agglutination test.

Another example of a lack of agreement in reciprocal tests may be noted in that the cells of *dussumieri* removed antibodies from the antiserum to *capicola* not only for themselves but also for those of *orientalis* (columns 4 and 6, line 2), and the cells of *orientalis* absorbed the antibodies for *dussumieri* as well as for themselves (columns 4 and 6, line 4). From these results, it would be concluded that, in relation to the specificities of the antibodies in *capicola* antiserum, the blood cells of *dussumieri* and *orientalis* share indistinguishable and presumably identical antigenic substances. The cells of these two species should then react alike with all other reagents prepared from *capicola* antiserum, and this expectation is realized in the pertinent data of the table. (Compare the reactivities in columns 4 and 6, lines 2 to 8, inclusive.) Furthermore, the reagents prepared from the antiserum to *capicola* following the respective absorptions of the cells of these two species (*dussumieri* and *orientalis*) should give parallel reactivities with the other test cells, and with but one exception this criterion was fulfilled (lines 2 and 4). The cells of *humilis* were agglutinated by the reagent prepared by absorbing this antiserum with *dussumieri* cells (column 5, line 2) but not by the reagent prepared by the absorption with the corpuscles of *orientalis* (column 5, line 4). The reciprocal of this latter reaction—anti-*humilis* serum absorbed with *orientalis* cells and tested with the cells of *capicola*—was not in agreement (column 3, line 18) and constitutes the second exception in the expected reciprocal reactivities (superscript 2). The lack of this latter agreement may be ascribed to the failure of *humilis* cells to be agglutinated by the reagent made by exhausting

capicola antiserum with *orientalis* corpuscles (column 5, line 4).

Barring the reciprocal removal of antibodies from anti-*capicola* serum for each other by the cells of *dussumieri* and *orientalis,* the reactions obtained following the respective absorptions by the corpuscles of the five other species revealed that each of these five species completely removed antibodies only for its own cells, leaving antibodies capable of various degrees of reactivity with the cells of each of the other species. *Capicola* therefore definitely possesses differentiating substances in contrast to each of the other species with which it has been compared. However, the differentiating substances of *capicola* demonstrated by these methods are shared, at least in part, by all other species tested than the one with which the comparison was made (i.e. the species whose cells were used in the absorption), except that *capicola* shares the same substances with both *dussumieri* and *orientalis.*

The recognition of the differentiating substances is based entirely upon the presence or absence of the agglutination of the cells of the various species by the respective reagents prepared as previously described. However, a suggestion of the degree of relationship can be inferred from the strength of the reaction of the cells of various species with a given reagent. Thus, the *capicola* cells were strongly or completely agglutinated by each of the various reagents prepared from the *capicola* antiserum, and the cells of the other species gave varying strengths of reactivities with each reagent. In general, the cells of *dussumieri, humilis,* and *orientalis* removed more antibodies from the antiserum to *capicola* than did those of Pearlneck, Ring dove, *semitorquata* and Senegal, as may be noted in table 1 from the strength of the reactions with the various reagents of the cells of the species in these two arbitrary groupings (compare the reactions of the cells in lines 2, 3 and 4 with those in lines 5, 6, 7 and 8). From these observations it can be inferred that *capicola* shares a greater number of these antigenic substances with *dussumieri, humilis* and *orientalis* than it does with Pearlneck, Ring dove, *semitorquata* and Senegal. Further, the cells of Ring dove and *semitorquata* are similar in their relationship to those of *capicola,* but are quite different in this respect from those of Pearlneck and Senegal. Since the absorption of *capicola* antiserum by the blood cells of Senegal removes nearly all the antibodies of Pearlneck cells (column 7, line 8), but the same degree of reciprocal relationship does not obtain (column 10, line 5), it can be inferred that *capicola* shares with Senegal nearly all of those antigens which *capicola* and Pearlneck have in common, plus one or more in addition.

Hence from the analysis of the specificities of *capicola* antisera as revealed by the results given in table 1, certain general conclusions may be made. First, *capicola* shares the same or nearly the same antigenic substances with both *dussumieri* and *orientalis,* and possesses specific substances not shared with either of these two species. A minor proportion of these specific substances of *capicola* (not in either *dussumieri* or *orientalis*) is shared with *humilis,* Pearlneck, Ring dove, *semitorquata,* and a larger proportion with Senegal, but it cannot be inferred from the present results whether the antigenic specificities which *capicola* shares with *humilis,* Pearlneck, Ring dove, *semitorquata* and Senegal, in contrast to *dussumieri* and *orientalis,* are identical, similar or different substances. Similarly, the data in table 1 (lines 3, 5, 6, 7 and 8) allow the conclusion that *capicola* shares with other species, in varying degrees, the cellular antigens which distinguish it from each species with which it is contrasted.

The second conclusion is concerned with the varying degrees of relationship to *capicola* which may be inferred from these tests. The basic assumption from previous work is that the cellular antigens of each species are gene-determined. It would follow that, by this criterion, the more cellular antigens which are shared

by two species, the closer would be their genetic relationship and the greater would be the proportion of antibodies removed from an antiserum to one species by the cells of the other than would obtain for other species sharing less antigenic substances. If, then, the degree of agglutination of the cells of various species given in table 1, following the respective absorptions of *capicola* antiserum, may be suggestive of the proportion of antibodies remaining in the antiserum for the cells of the species, it becomes possible to assess the relative degree of relationship of the other species to *capicola*. The cells of *dussumieri, humilis* and *orientalis* removed more antibodies from anti-*capicola* serum than did those of the other species, according to the strength of the agglutination reactions of the respective reagents with the cells of the other species. Hence, by this criterion *dussumieri, humilis* and *orientalis* are a "group" of species more closely related to *capicola* than are Pearlneck, Ring dove, *semitorquata* and Senegal. Ring dove and semitorquata form a second "group" of intermediately related species to *capicola*, since the cells of each remove more antibodies for the cells of the other than for those of the other species. Pearlneck and Senegal comprise another "group" possibly slightly less closely related to *capicola* than are Ring dove and *semitorquata*, but Senegal is more closely related to *capicola* than is Pearlneck (compare the reactions in lines 5 and 8).

Relationships of Pearlneck to Other Species

It would be possible, in analyzing the data of table 1, to consider separately the specificities of the respective antisera, as has been done above for *capicola* antiserum. However, what appears to be more pertinent is to examine in some detail the interrelationships of the antigenic specificities of a single species, as Pearlneck, for which there is a considerable body of information regarding the genetic interrelationships of its cellular antigens. It may be inferred that similar patterns of relationships will obtain for the other species of this study, and probably for species relationships in general. The interrelationships of the cellular antigens of Pearlneck to those of *dussumieri, humilis*, Ring dove and Senegal will therefore be examined in detail.

It may be noted in table 1 (lines 30 to 36 inclusive) that, with but one exception, the absorption of Pearlneck antiserum by the blood cells of each of the other seven species resulted in complete removal of antibodies only for the absorbing cells at the absorbing (1:60 or 1:90) and higher dilutions, leaving antibodies reactive in various degrees of strength with cells from the other species. The exception is that the absorption of Pearlneck antiserum by Senegal cells removed antibodies completely or nearly completely for Ring dove cells (column 8, line 36) from which it may be inferred that Pearlneck shares also with Senegal all or nearly all the antigenic substances which it shares with Ring dove.

The relationship of the antigenic components of the cells of Pearlneck in contrast to those of Ring dove (line 34, columns 7 and 8) may be represented diagrammatically, as in figure 1. The space in figure 1 arbitrarily assigned to the antigenic characters of Pearlneck, in contrast to Ring dove, is composed of at least nine cellular characters that have been obtained separately (Irwin, 1939). These are called d-1, d-2, d-3, d-4, d-5, d-6, d-7, d-11 and d-12. There may of course be substances other than these nine which set Pearlneck apart from Ring dove but these are not presently recognized. The area arbitrarily assigned in figure 1 to the antigenic substances which are shared by Pearlneck with Ring dove is an index of the extent to which Pearlneck and Ring dove possess some identical and some similar antigenic substances, and presumably, therefore, of the extent to which the two species possess identical and similar genes with effects on the cellular antigens. The same kind of diagram would portray

FIG. 1. Diagram of the cellular antigens of Pearlneck, showing the proportions (assigned arbitrarily) of those specific to Pearlneck to those shared with Ring dove.

the relationships between Pearlneck and any other species of those listed in the table, although the proportionate amounts of *specific* and *common* components might vary from those assigned in figure 1.

Since the reagent resulting from the absorption of Pearlneck antiserum by Ring dove cells contains antibodies reactive with the cells of each of the other species as well as with those of Pearlneck (line 34), it is clear that one or more of the nine antigenic substances peculiar to Pearlneck must be shared with the other species either as identical or similar substances. However, the same substances, or the same similar substances, of Pearlneck, need not appear in each of the other species. The data presented in a previous paper (Irwin, 1953) provide evidence that the six species of the present study, other than Pearlneck and Ring dove, share one or more of these nine antigenic characters present in Pearlneck and not in Ring dove. These data are summarized as follows:

Species	Antigenic characters shared with Pearlneck as	
	Similar substances	Identical substances
capicola	d-1, d-3, d-11	
dussumieri	d-1, d-2, d-3, d-11, d-12	d-4 (?)
humilis	d-1, d-11, d-12	d-4
orientalis	d-1, d-11	d-4
semitorquata	none recognized	none recognized
Senegal	d-1, d-2, d-6, d-7, d-11	d-3, d-4, d-5, d-12

Another method of determining whether the cellular antigens of Pearlneck, in contrast to Ring dove, are the same or different in each of the other species under test, is to use the cells of each species in combination with those of Ring dove in absorption of Pearlneck antiserum and to test the respective reagents so produced with the cells of the different species. Such tests are given in table 2. The data of table 2 and pertinent data of table 1 can be utilized in combination to give precision to further diagrammatic representations of the cellular antigens of Pearlneck as these may be present in the other species.

For example, the sharing and non-sharing of the cellular antigens of Pearlneck with either or both *dussumieri* and Ring dove are represented in figure 2. In table 2, it may be seen (line 2) that the cells of *dussumieri* are agglutinated at a high titer (7th and 8th dilutions, or 1:5760 or 1:11,520) by the reagent for Pearlneck specific substances in contrast to Ring dove (see also table 1, line 34). Further, the reagent made by using the cells of *dussumieri* and Ring dove together in saturation of Pearlneck antiserum (table 2, line 4) was weakly reactive with the cells of *humilis, orientalis,* and *semitorquata,* and strongly reactive with those of *capicola* and Senegal, as well as Pearlneck. In general, no combination of the cells of Ring dove and another species in absorption of Pearlneck antiserum removed completely antibodies for the cells of any other species, except that both *capicola* and *orientalis* removed all or nearly all the antibodies for the cells of *semitorquata* (table 2, lines 3 and 6). Otherwise, there was observed only a definite reduction in the degree of reactivity of the cells of some other species following such absorp-

TABLE 2. *Interrelationships of the cellular antigens of Pearlneck with those of other species of Streptopelia*

Pearlneck antiserum absorbed by the erythrocytes of	Agglutinations of the blood cells of various species of Streptopelia with reagents made from Pearlneck antiserum following absorptions of antibodies by various combinations of the cells of the other species: Test cells								Line
	capicola	*dussumieri*	*humilis*	*orientalis*	Pearlneck	Ringdove	*semitorquata*	Senegal	1
Ring dove	5, 6, 7	7, 8	7, 8	6, 7	8, 9	0	1, 2	7, 8	2
Ring dove and *capicola*	0	++	++	+	C	0	?	++	3
Ring dove and *dussumieri*	++	0	±	±	C	0	±	++	4
Ring dove and *humilis*	++	+	0	+	C	0	±	++	5
Ring dove and *orientalis*	++	+	+	0	C	0	?	++	6
Ring dove and *semitorquata*	++	++	++	++	C	0	0	++	7
Ring dove and Senegal	++	++	++	++	C	0	±	0	8
capicola and *dussumieri*	0	0	±	+	C	0	±	C	9
dussumieri and *humilis*	±	0	0	0	C	0	±	C	10
dussumieri and *orientalis*	?	0	0	0	C	?	±	C	11
dussumieri and Senegal	0	0	0	0	C	0	0	0	12
humilis and *orientalis*	+	++	0	0	C	0	±	C	13
humilis and Senegal	±	+	0	+	C	0	0	0	14
Column 1	2	3	4	5	6	7	8	9	

The digits represent the highest dilution of the reagent at which agglutination was visible. Thus, if the first dilution of the reagent-cell mixture was 1:90, 1 = 90, 2 = 180, 3 = 360 . . . 9 = 23,040.

Symbols: See table 1.

tions, indicating that certain antigens of Pearlneck were shared at least in part by two or more of the species. Thus, Pearlneck shares with *dussumieri* an appreciable proportion of the antigenic substances which distinguish it from Ring dove, primarily if not entirely by virtue of antigenic substances in *dussumieri* similar to d-1, d-2, d-3, d-11 and d-12, and a substance identical to d-4 of Pearlneck. Pearlneck also possesses a considerable proportion of cellular antigens specific to itself which is not shared with either Ring dove or *dussumieri*. These relationships are diagrammatically presented in figure 2.

Further, the data of table 1 (column 8, line 31) show that Pearlneck shares with Ring dove only a minor fraction of its antigenic components which are not also shared with *dussumieri*, since Ring dove cells are only weakly agglutinated by the test-fluid following absorption of Pearlneck antiserum with the cells of *dussumieri*. By inference, then, the major portion of the antigenic components of Pearlneck which are common to Ring dove are also shared with *dussumieri*. Thus in figure 2 it is shown that the major proportion of the antigenic substances of Pearlneck which are held in common with Ring dove is also shared with *dussumieri*, and that Pearlneck shares with Ring dove only a minor fraction of substances to the exclusion of *dussumieri*.

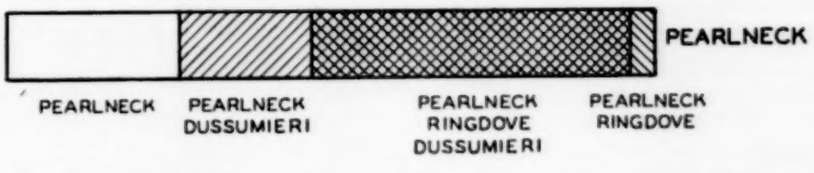

FIG. 2. An analysis of the cellular antigens of Pearlneck, showing the relative proportions of those which are shared with either *dussumieri* or Ring dove, or both.

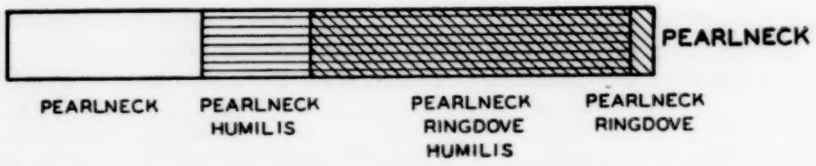

FIG. 3. An analysis of the antigenic components of the blood cells of Pearlneck, showing the interrelationships with *humilis* or Ring dove, or both.

By the same kind of analysis of the interrelationships of the cellular components of Pearlneck in contrast to *humilis* and Ring dove, the proportions of cellular antigens as they are specific to Pearlneck, or are shared with either *humilis* or Ring dove, or both, are presented diagrammatically in figure 3. The data in table 1 (column 8, line 32) show that Pearlneck shares with Ring dove a minor proportion of cellular components to the exclusion of *humilis,* since absorption with *humilis* cells did not remove all the antibodies for Ring dove cells. By inference, the greater part of the substances of Pearlneck which are shared with Ring dove is also shared with *humilis*. Further, from the data of table 2 (column 4, line 2), it is evident that Pearlneck shares with *humilis* an appreciable proportion of the antigenic characters which are peculiar to itself in contrast to Ring dove, and that Pearlneck possesses certain antigens to the exclusion of both *humilis* and Ring dove (column 6, line 5). The antigenic substances of *humilis* which are known to be shared with Pearlneck are those which are antigenically similar to the d-1, d-11 and d-12, and seemingly identical with d-4, of Pearlneck, as given above. The proportions of these four divisions of the cellular antigens of Pearlneck, in relation to Ring dove and *humilis,* are given in figure 3.

Similarly, from the data in table 1 (line 36) and table 2 (lines 2 and 8), it is possible to represent diagrammatically the interrelationships of the cellular antigens of Pearlneck to those of Ring dove and Senegal, as are given in figure 4. Senegal cells remove antibodies from Pearlneck antiserum for Ring dove cells as well as for themselves (column 8, line 36), indicating that all the cellular antigens which are shared by Pearlneck and Ring dove are also present in Senegal. This result has been obtained with all antisera to Pearlneck with which such tests have been made. Reciprocally, one antiserum to Ring dove when saturated with Senegal cells was weakly reactive with Pearlneck cells and has been so reported (Irwin and Cole, 1940), although other antisera to Ring dove have not shown this specificity (table 1, column 7, line 43). It appears that the fraction of antigenic constituents shared by Pearlneck and Ring dove, to the exclusion of Senegal, if any exists, is very minor in proportion, and therefore no such area is shown in figure 4. As has been reported elsewhere (Irwin, 1951) Pearlneck shares with Senegal as identical substances four (d-3, d-4, d-5, and d-12) of the nine substances which differentiate Pearlneck from Ring dove, and as similar substances the remaining five (d-1, d-2, d-6, d-7, and d-11). Thus the proportion of specific Pearlneck substances shared with Senegal, in contrast to Ring dove, is undoubtedly greater than that shared with any other species tested. That is, the

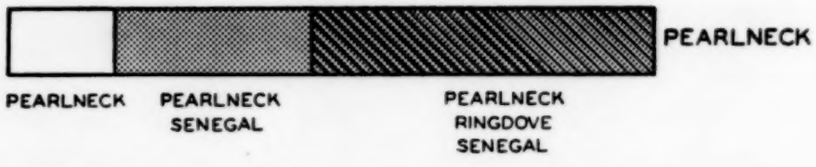

FIG. 4. A diagrammatic representation of the interrelationships of the cellular antigens of Pearlneck with either Ring dove or Senegal, or both.

FIG. 5. A diagrammatic representation of the cellular antigens of Pearlneck, depicting the relative proportions which are species-specific or are shared with *dussumieri, humilis,* Ring dove and Senegal. Area 1 = Pearlneck only; area 2 = Pearlneck and *dussumieri;* area 3 = Pearlneck, *dussumieri* and *humilis;* area 4 = Pearlneck, *dussumieri, humilis* and Senegal; area 5 = Pearlneck and Senegal; area 6 = Pearlneck, *dussumieri* and Senegal; are 7 = Pearlneck, *dussumieri, humilis,* Ring dove and Senegal; area 8 = Pearlneck, *dussumieri,* Ring dove and Senegal; area 9 = Pearlneck, *humilis,* Ring dove and Senegal.

cellular antigens of Pearlneck are more closely related to those of Senegal than to those of any of the other seven species of Streptopelia.

Figure 5 depicts a diagrammatic representation of the cellular antigens of Pearlneck as these are peculiar to Pearlneck or are shared in various combinations with *dussumieri, humilis,* Ring dove and Senegal. Dealing first with those antigens shared by Pearlneck and Ring dove, it has been stated that these are also shared with Senegal, since exhaustion by Senegal cells leaves no antibodies in Pearlneck antiserum for Ring dove cells (table 1, column 8, line 36). Both *dussumieri* and *humilis* also contain the majority of these common substances, and area 7 of figure 5 depicts the proportion of the antigenic substances of Pearlneck which are shared by the five species. Neither *dussumieri* nor *humilis* contains all the antigenic substances which Pearlneck shares with Ring dove, since the cells of neither species removed the antibodies for Ring dove cells from Pearlneck antiserum (table 1, column 8, lines 31 and 32). The test of the reciprocal reactivities was in agreement since anti-Ring dove serum absorbed with the cells of either *dussumieri* or *orientalis* was reactive with Pearlneck cells, as is given in table 1 (column 7, lines 38 and 39). Since the cells of *dussumieri* and *humilis* in combination absorb from Pearlneck antiserum the antibodies for Ring dove cells (table 2, column 7, line 10) whereas the cells of neither species are able to do this by themselves, it appears justifiable to conclude that each of these two species shares with Pearlneck a minor but significant and different fraction of the antigens that Pearlneck shares with both Ring dove and Senegal. These specific proportions are portrayed in areas 8 and 9 of figure 5.

It has already been stated that, of the nine cellular antigens which are peculiar to Pearlneck in contrast to Ring dove, four (d-3, d-4, d-5 and d-12) are present in identical form in Senegal, and the remaining five (d-1, d-2, d-6, d-7 and d-11) are found in Senegal as related substances. Therefore, any parts of these nine substances which Pearlneck may share with *dussumieri* or *humilis* to the exclusion of both Senegal and Ring dove must be a part or parts of one or more of the latter five substances, or of others not now recognized. The data in table 1 (columns 4 and 5, line 36) and in table 2 (columns 3 and 4, line 8) show that the reagent prepared by the saturation of Pearlneck antiserum with Senegal cells contained strongly reacting antibodies for the corpuscles of both *dussumieri* and *humilis.* Thus it seems clear that these two species share with Pearlneck a different part or parts of one or more of the five antigenic substances (d-1, d-2, d-6, d-7 and d-11) which Pearlneck and Senegal share only as related antigenic characters, or possibly of one or more antigenic characters of Pearlneck not yet detected.

It may be noted in table 2 (line 2) that there is a high titer of the cells of each of these two species (*dussumieri* and *humilis*) with the reagent for Pearlneck specific substances not in Ring dove. Following the respective absorptions of the

antiserum to Pearlneck by the cells of either species, *dussumieri* or *humilis,* in combination with those of Ring dove, the titer of each resulting reagent for cells of the other species was sharply reduced, as is given in table 2 (columns 3 and 4, lines 4 and 5). From these results one may conclude that Pearlneck shares more of its specific substances, not shared with Ring dove, with both *dussumieri* and *humilis* than it shares with either species by itself, as is depicted in area 3, of figure 5. Pearlneck shares all or nearly all of d-4 with both *dussumieri* and *humilis* as well as with Senegal, and possibly parts of d-1, d-11 and d-12, and these antigens are represented in area 4 of figure 5.

One may deduce from the data of table 2 that Pearlneck shares with *dussumieri* a part or parts of one or more antigenic substances which are different from those shared with both *humilis* and Senegal, since the saturation of Pearlneck antiserum with the cells of both *humilis* and Senegal did not remove all the antibodies for the cells of *dussumieri* (table 2, column 3, line 14). This common antigenic specificity of Pearlneck and *dussumieri* is illustrated in area 2 of figure 5. At present it is not possible to deduce which of the known antigenic constituents of Pearlneck is involved, except that it must be a part or parts of d-1, d-2, or d-11, since d-3 and d-12 are shared *in toto* with Senegal.

On the other hand, a comparable absorption with the cells of both *dussumieri* and Senegal (table 2, column 4, line 12) removed the antibodies not only for the cells of *humilis,* but for those of all the other species tested as well, except for those of Pearlneck. The reactivity of Pearlneck cells appears to be primarily, but not necessarily entirely, concerned with the d-11 antigen. Thus it appears that *dussumieri* and Senegal together possess all the antigenic components of Pearlneck which are shared by any of the other species used in these comparisons. Pearlneck therefore possesses a part or parts of one or more antigenic substances which it does not share with any of the other seven species of Streptopelia, as is pictured in area 1, figure 5.

Also, Pearlneck and Senegal share an appreciable proportion of antigenic characters not found in any of the other species, since the exhaustion of antibodies in Pearlneck antiserum by the cells of both *dussumieri* and *humilis* (table 2, line 10) removes antibodies for the cells of these two species, for those of *orientalis* and Ring dove, and leaves strongly reacting antibodies for both Pearlneck and Senegal cells. This proportionate relationship of the cellular antigens of Pearlneck to those of Senegal is shown in area 5 of figure 5.

It is not possible to determine directly from the data of tables 1 and 2 whether Pearlneck shares any part of its cellular antigens, not shared with Ring dove, with Senegal and either *dussumieri* or *humilis* to the exclusion of the other. However, the data given in a previous paper (Irwin, 1953) showed that *dussumieri* contains cellular substances related to the d-1, d-2, d-3, d-11 and d-12 of Pearlneck whereas *humilis* contains cellular components related to d-1, d-11 and d-12. Senegal possesses in common with Pearlneck all of d-3 and d-12, and substances related to d-1, d-2 and d-11 (Irwin, 1951). Pearlneck may share with Senegal and with either *dussumieri* or *humilis* a part of d-1 or d-11 which is not held in common with the other of these two species. Further, Pearlneck may share with both Senegal and *dussumieri* a part of d-2. It appears reasonable, on the basis of this evidence, to ascribe tentatively a minor proportion of Pearlneck antigens to be held in common with *dussumieri* and Senegal, to the exclusion of both *humilis* and Ring dove. This relationship is depicted in area 6 of figure 5.

The analysis of the antigenic components of Pearlneck cells allows their division into two main groups; (a) those shared by Pearlneck with Ring dove, and (b) those peculiar to Pearlneck in contrast to Ring dove. The majority if not all of

these latter substances have been obtained as nine or more unit-substances in back-cross hybrids. Pearlneck shares with Senegal all or nearly all the common substances of Pearlneck and Ring dove, four (d-3, d-4, d-5 and d-12) of the nine substances which differentiate Pearlneck from Ring dove, and a part or parts of each of the other five (d-1, d-2, d-6, d-7 and d-11) antigenic characters. Those substances peculiar to Pearlneck to the exclusion of these four other species must be parts of one or more of those five substances, primarily d-11, as shown in area 1, figure 5. Pearlneck possesses in common with *dussumieri* a part or parts of one or more antigenic substances not shared with *humilis,* Ring dove or Senegal (fig. 5, area 2), and antigenic specificities with both *dussumieri* and *humilis* to the exclusion of both Ring dove and Senegal (fig. 5, area 3). There is almost indisputable evidence that Pearlneck shares with *dussumieri, humilis* and Senegal, to the exclusion of Ring dove, one or more antigenic substances or parts thereof (fig. 5, area 4). The d-4 substance of Pearlneck is present in each of these species in presumably identical form, and parts of other antigenic substances of Pearlneck may also be shared by these three species to make up area 4 in the diagram. There exists also a part or parts of several specific components of Pearlneck, in comparison with Ring dove, which are shared only with Senegal (area 5, fig. 5). The substance d-5 of Pearlneck is shared entirely or nearly so only with Senegal, and Pearlneck also shares with Senegal parts of d-6 and d-7 which seemingly are not present at all in any other species tested of *Streptopelia.* These substances of Pearlneck make up a part of area 5. An area for which there is very little direct evidence is that which is shared by Pearlneck with both *dussumieri* and Senegal (area 6), to the exclusion of *humilis* and Ring dove.

Of the substances of Pearlneck which are shared by Ring dove, the data indicate that all or nearly all are also shared with Senegal, and that nearly all these common substances are also shared with both *dussumieri* and *humilis.* The areas 7, 8 and 9 of figure 5 depict the relative proportions of these relationships, with area 7 being assigned to those substances shared by Pearlneck with the other four species, and with only a very small proportion of antigenic specificities shared respectively by (a) Pearlneck with *dussumieri,* Ring dove and Senegal (area 8), and (b) with *humilis,* Ring dove and Senegal (area 9). The only substance shared by Pearlneck and Ring dove, and therefore with Senegal, which has been identified as a genetic unit is a substance (A) peculiar to *C. guinea* in contrast to *C. livia* (Irwin, 1938), which probably forms a part of area 7.

As was stated previously, this diagrammatic representation of the relationships of the antigenic substances of Pearlneck to those of the four of the other species of Streptopelia used in these tests would be more or less paralleled if the antigenic components of the blood cells of any of the other species were analyzed in this manner. The general picture that emerges is one of various degrees of interlocking relationships of (heritable) antigenic characters when the antigens of any one species are compared with those of several other species by the method of antibody-absorption of the antiserum to a given species. Presumably the same kind of relationships may be deduced among the genes producing these antigenic substances that exist for the cellular antigens, which of course are gene products.

It appears reasonable to propose that the antigenic substances *common* to these eight species, which are represented diagrammatically for five species in figure 5 (area 5), are produced by genes which are more primitive than are those which effect the differentiating substances of the various species. One index of the existence of individual entities among these common antigenic substances is that provided in a report by Buchbinder (1934) who noted that an antigen of a strain of

Pasturella would induce antibodies which were reactive with red blood cells of many species of Columbiformes, including Pearlneck and Ring dove, presumably by virtue of an identical substance in the various genera tested. Further, results as yet unpublished from this laboratory (by W. J. Miller and the writer) have demonstrated that each of the eight species of Streptopelia possesses on its cells as identical or similar substances certain of the antigenic characters by which *Columba guinea* is distinguished from the domesticated form of *C. livia*.

The identification of the respective antigenic characters which differentiate Pearlneck from Ring dove, and the presence or absence of these in the other species as presumed identical, or as similar substances, permit an estimate of the primitiveness of the causative genes. Referring to the summary given earlier of data from a previous paper (Irwin, 1953) the d-4 substance of Pearlneck is identical in five of these eight species of Streptopelia but was not detected in the other three (*capicola,* Ring dove and *semitorquata*). Further, substances similar to d-1 and d-11, respectively, are present in five species (*capicola, dussumieri, humilis, orientalis* and Senegal), but not necessarily as the same similar substances in each of these species, and absent in the other two (Ring dove and *semitorquata*). Hence the causative genes for d-1, d-4 and d-11, respectively, would be somewhat less primitive than are those which produce the common antigenic substances of the eight species. Only two species (*dussumieri* and Senegal) possess an antigenic substance similar to the d-2 of Pearlneck, and Senegal possesses d-3 and d-12 as identical substances, while *capicola* contains a substance similar to d-3 and substances similar to both d-3 and d-12 are present in *dussumieri*. The d-5 antigen of Pearlneck is presumably identical, or nearly so, in Senegal, and substances similar to the d-6 and d-7 were detected only in Senegal. Hence the gene or genes for the respective substances of Pearlneck which distinguish it from Ring dove may be placed in a descending order of primitiveness—d-4, d-1 and d-11, d-3 and d-12, d-2, d-5, d-6 and d-7.

Other Relationships

The eight species of Streptopelia under study appear to form two and possibly three reasonably well-defined groups, as may be inferred from the reactions given in tables 1 and 2. For example, Ring dove and *semitorquata* seemingly are much more closely related to each other than either is to any other species. Thus, when the cells of either Ring dove or *semitorquata* were used in absorbing the antibodies in the antisera to any one of the six other species, antibodies only faintly reactive with the cells of the other of these two species were left in the antiserum, if any at all remained (compare the reactions of the cells of these two species in table 1, columns 8 and 9, lines 6 and 7, 13 and 14, 20 and 21, 27 and 28, 34 and 35). Also, the cells of either of these two species in absorption of the antiserum to the other removed nearly all the antibodies from the antiserum (table 1, lines 42 and 49), in contrast to the strong reactivities for the cells of both Ring dove and *semitorquata* following absorptions of either antiserum by the cells of any of the other species (lines 37 to 50 inclusive). These results indicate very clearly that these two species differ very little from each other in their content of cellular antigens. Therefore, it may be concluded that they also differ very little in the genes producing the cellular antigens. A very pertinent question is how different these two species might be in respect to genes acting on the antigens of other tissues and fluids.

Mention has already been made of the close relationship of the cellular antigens of Pearlneck and Senegal (Irwin, 1951). It may also be noted in table 1 that, whenever Senegal cells were used in the absorption of the antisera to the other species, the reactivities of the resulting

reagents for Pearlneck cells were either very weak or non-existent (column 7, lines 8, 15, 22, 29, 43 and 50). On the other hand, following absorptions of the same antisera with Pearlneck cells, the respective reagents reacted strongly with the cells of Senegal (column 10, lines 5, 12, 19, 26, 41 and 48). Therefore if any difference exists between the relative relationships of Pearlneck and Senegal to the other six species, it appears that Senegal occupies a position somewhere between each of the other species and Pearlneck.

The remaining four species, *capicola, dussumieri, humilis* and *orientalis,* appear in general to be more closely related to each other than any one of them is to either Pearlneck, Ring dove, *semitorquata* or Senegal. Thus in table 1 it may be seen that the absorption of the antiserum to any one of these four species by the cells of any other of the four removed a greater proportion of antibodies than did the cells of Pearlneck, Ring dove, *semitorquata,* or Senegal. By the same line of reasoning it appears that the results given in table 1 with the reagents from both Ring dove and *semitorquata* antisera, prepared by absorption with the cells of the other species, suggest that these two species are less closely related to either Pearlneck or Senegal than to the other four species. That is, the cells of Pearlneck or Senegal did not remove as many antibodies from either of these two antisera (lines 41, 43, 48 and 50) as did those of the other four species (*capicola, dussumieri, humilis* and *orientalis*).

Thus it appears that, by the criterion of relationships of their cellular antigens, Pearlneck and Senegal form one group of closely related species within the genus, Ring dove and *semitorquata* are another. The remaining four species are somewhat more closely related to each other than to the species in either of the other two groups, and seemingly are related slightly more closely to Ring dove and *semitorquata* than to Pearlneck and Senegal.

The members of each of these three groupings do not appear to owe their relative relationships to proximity of geographic range. For example, Pearlneck is indigenous to southern China and surrounding areas and Senegal to various regions of Africa. Further, if Ring dove is a descendant of the wild species *roseogrisea* which is indigenous to Africa, there would appear to be a considerable overlapping of its geographic range with that of *semitorquata,* to which it is closely related antigenically. And Pearlneck is seemingly less closely related antigenically to *dussumieri, humilis* and *orientalis,* which are native to regions more or less overlapping the range of Pearlneck, than is Senegal, which is native to Africa. It was previously reported (Cumley and Irwin, 1944) that the cellular antigens of 11 species of Columba from either the Old or New World, respectively, were more like those of any other species of the particular hemisphere than they were like any single species of the other. No parallel association of geographic range and degree of relationship of the cellular antigens appears to obtain in the Streptopelia.

Discussion

Considerable attention in various laboratories has been given in recent years to assembling data which indicate that related species have many homologous genes. The genetic point of view concerning related species has been aptly stated by Morgan, Bridges and Sturtevant (1925) that "Related species must have many genes in common—they are in fact similar only because of this group of common genes." Leading references to papers on this subject were given in a previous paper (Irwin, 1953), and will not be repeated here. Special attention should be called, however, to the volume by Patterson and Stone (1952) which appeared after the previous paper had been accepted for publication. In this classical publication on evolution in the genus Drosophila, the authors not only have brought together

the present information on evolution in this genus, but have made pertinent comparisons with, and citations of, many studies on evolutionary patterns in both plants and animals.

The interpretation of the evidence presented in the present paper of antigenic interrelationships of the erythrocytes among eight species of Streptopelia depends for its genetic implications upon previous data (for a review, see Irwin, 1951) obtained from a study of the offspring of species hybrids and backcross hybrids of crosses of three of the species—Pearlneck, Ring dove and Senegal. Both Pearlneck and Senegal differ from Ring dove in that one or more genes on nine or ten chromosomes effect respective cellular antigens which are illustrative of qualitative differences between each of these species and Ring dove. Furthermore, four of nine such antigenic characters of Pearlneck are indistinguishable from, and presumably identical with, four substances of Senegal, implying identity of the causative genes. The other five substances of Pearlneck are similar to, but not identical with, five antigenic substances of Senegal, and it may be inferred that the causative genes in the two species for these respective substances are similar but not identical. One heritable antigen of Senegal has not been demonstrated to be related to any in Pearlneck, and presumably the causative gene or genes are not related to any in Pearlneck. It would seem reasonable to anticipate that the number of cellular antigens held in *common* by any pair of these three species would be at least equal to, and probably greater than, the number of those which differentiate each pair, but there is no pertinent information as to whether the causative genes for the common antigens are located on the same chromosomes as those which effect the differentiating substances, or on others. The only available information of this sort is that the causative genes for antigenic substances identical in Pearlneck and Senegal, which in turn distinguish each species from Ring dove, are on different chromosomes than are those genes which have antigenic products which differentiate these two species from each other and also from Ring dove.

If to the number of chromosomes carrying genes effecting cellular antigens which differentiate one species from another are added those with genes effecting the antigens of the serum, of which there are at least four differentiating these species (Cumley and Irwin, 1952), slightly less than half the probable number of chromosomes of these species will carry genes with effects differentiating the constituents of the blood of either Pearlneck or Senegal from that of Ring dove. (The number of chromosomes in Pearlneck and Senegal is probably the same as has been reported for Ring dove and the domesticated pigeon (*Columba livia*) by Painter and Cole (1943), in which the haploid number is 34 ± 2.) One cannot, of course, ignore or exclude the possibility that other antigenic substances which differentiate either species from Ring dove may exist and are not yet detected. However, if there are many of these, it appears that they are not demonstrable at the concentrations of antisera used in these tests. No claim is advanced that *all* the differentiating antigenic substances of the blood in any comparison of a pair of species have been detected, but it is probable that most of them are recognized.

These comparisons have necessarily been limited to the bloods of a relatively small number of individuals within each species. How far antigenic variation between individuals within any of these species may be found to alter the distinction between the species is an unanswered question. Some individual differences within certain species have been noted (unpublished observations) which are detectable at the level (dilution of antiserum, 1:60) at which the tests herein reported were made, but these appear to be exceptional rather than general. In the light of the previous experience in this laboratory, it is questionable if the individual differences that undoubtedly exist

within the various species affect appreciably the general pattern of antigenic relationships among the species as described in this study.

One general conclusion from these studies is that a proportion of the antigens of the blood (cells and serum) represent qualitative differences between species, and that the causative genes for these differences may be located on half or less of the chromosomes. In contrast, the analysis of data, as yet unpublished, on several morphological characters of these species (from measurements taken under the supervision of the late Professor L. J. Cole) points to the conclusion that the average value for each of these morphological characters in the species hybrids and backcross populations is as expected if multiple genes with individually small effects were involved. (The details of these latter studies will be published elsewhere.) Thus the explanations of the respective genetic bases for the antigenic and morphological characters of the same experimental material appear to be somewhat at variance.

A pertinent question concerning the antigenic relationships among these species is whether the antigenic substances which differentiate one species from another are the respective products of single genes, or of linked genes. If an antigenic substance differentiating two species, as the d-4 in Pearlneck, is the end-product of but one gene, any substance in another species which is similar to d-4 would presumably represent the action of a gene similar to the one in Pearlneck effecting d-4, and the relationship of the causative genes in the two species would undoubtedly be that of alleles. Under such conditions the differences between species could be of the same order as individual differences within species, such as the A and B substances of man, or any of the contrasting cellular antigens in man or other animal species. Thus, since in either man (Race and Sanger, 1950; Wiener, 1943) or cattle (Stormont, Owen and Irwin, 1951; Stormont, 1950) one or more cellular antigens in at least ten genetic systems are demonstrable, it would be theoretically possible within either species to obtain by selection the respective classes of individuals which would differ in the end products of genes in each of the ten or more genetic systems. Would the antigenic differences between these two hypothetical classes be of the same order as those between Pearlneck and Ring dove, which are differentiated by cellular antigens in ten genetic systems? If so, the differentiation of cellular antigens between these species is of the same order which might be found within species.

However, fractionations of the d-4 substance of Pearlneck have been noted (Irwin, 1951) which presumably were the result of crossovers of causative genes, indicating that this substance is the result of the joint action of two or more linked genes which rarely show crossovers in the backcross birds, in which the other chromosome of the pair is from Ring dove. As stated above, the d-4 substance is found as an indistinguishable and presumably homologous substance in *dussumieri, humilis, orientalis* and Senegal, implying linked genes in these other four species, as in Pearlneck. One cannot ignore the possibility that the other substances of Pearlneck—in contrast to Ring dove—are also the products of two or more genes, but experimental evidence for any such assumption is lacking.

If two or more linked genes are involved in the production of many or all species-specific antigenic substances, as has been observed for the d-4 substance, the explanation of the relationships of the causative genes in species with similar antigens is more involved than if each substance were the product of a single gene. The linked genes in one species could then have an effect only similar to that of the respective effects of the genes in the other species, and the relationships among the genes would be that of alleles. Another possibility would be that one or more such genes in each species would effect identical substances—thereby ac-

counting in part for their resemblance—and one or more other (linked) genes might effect either (a) antigenic substances similar in greater or less degree, or (b) substances with no similarity. The relationship of the causative genes under (a) would presumably be that expected of alleles, whereas that under (b) might be an example of changes in genes which were drastic enough so that they might no longer be classified as alleles.

Only suggestive evidence is available at present that the antigenic substances which differentiate one species of this study from another are produced by the joint action of linked genes. If additional evidence on this point should be obtained, there would still remain the question concerning the grouping of these genes on less than half the chromosomes of the species. In contrast, the explanation of the differences in morphological characters between Pearlneck and Ring dove, Senegal and Ring dove, or Pearlneck and Senegal, as stated above, seemingly agrees with the views held by many geneticists on the nature of genetic differences between species. This view has been expressed by Muller (1940) that such differences depend upon multiple genes, having individually small effects.

One primary conclusion which can be drawn from this study is that each of the species studied, in its antigenic interrelationships with the others, appears definitely to be an entity. The majority of the cellular antigens of a particular species appear to be shared, in various combinations, with other species—the closer the relationship between species, the more cellular antigens are shared. The cellular antigens which differentiate one species from another may be shared, at least in part, by one or more other species. The converse situation also obtains, that some of the cellular antigens which are common to a pair of species may serve to differentiate them from one or more other species. (Parallel relationships of course are ascribed to the causative genes as are described for the cellular antigens.) In addition to these interlocking relationships of the cellular antigens, each species may have specificities of one or more antigenic substances which are not shared by any other species, thus differentiating the species from all others. Thus Pearlneck differs from all other species tested in one or more antigenic specificities, primarily of the d-11 substance. But it is still an open question whether other species than those tested would possess on their cells the antigenic specificities of the d-11 substance which now set Pearlneck apart from the other species of this study.

In brief, these studies indicate that these eight species of Streptopelia are made up primarily of different combinations of cellular antigens, therefore of the causative genes. The interlocking relationships of the cellular antigens of a species, as of Pearlneck, with those of other species (see fig. 5), are an index of the changes in genes effecting cellular antigens which have taken place during the evolution of these species. It seems reasonable to propose that the differences in the gene or genes effecting the antigenic substances of the blood which differentiate a species from the others, and possibly also those effecting the antigens of either cells or serum by which one species is distinguished from another, are examples of Dobzhansky's (1941) statement that "Divergence of evolutionary lines . . . probably involves . . . a gradual increase of the differences between the structure of the genes which had been identical or similar in the ancestral forms."

Summary

An examination of the relationships of the cellular antigens of eight species of Streptopelia reveals primarily a mosaic of interlocking relationships of the antigenic substances of each species with those of the others. These have been presented diagrammatically for one species (Pearlneck) for which the cellular antigens specific to it in comparison with Ring dove

have been isolated as units in the backcross populations, following an original cross with Ring dove. On the basis of relative relationships of their cellular antigens, these eight species could be assigned to three fairly well-defined groups. Each species probably possesses one or more antigenic substances with specificities which distinguish it from all the other species. Presumably the relationships of the genes effecting the cellular antigens in the respective species parallel the relationships of the antigenic substances.

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ON THE TWO FORMS MACROGYNA AND MICROGYNA OF THE ANT *MYRMICA RUBRA* L.

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Introduction

Two forms of the Eurasian ant *Myrmica rubra* L. have been described (Brian and Brian, 1949) with the following differences:

macrogyna: females and males larger, the largest sexual females with the head dark brown above and orange-brown below the eyes, with longer, more tapering ovarioles, of which the terminal filaments account for only one-third the total length in virgins, eyes less prominent but of the same size, colonies usually monogynous with relatively large aggressive workers, the average worker head-width less than the average female head-width, colony reproduction by dissemination of fertile females which found colonies either alone or in small aggregates;

microgyna: females and males smaller, the smallest sexual females with head brown above and yellow-brown below the eyes, with shorter, less tapering ovarioles of which the terminal filaments account for two-fifths of the total length in virgins, eyes more prominent but of the same size, colonies polygynous with relatively small and docile workers, the average worker head-width similar to the average female head-width, fertile females return to their nest (and possibly other nests) and colonies reproduce by fission.

To this can now be added: *microgyna* queens are unable to found colonies alone (as can *macrogyna*) even under laboratory conditions; [2] that whereas *macrogyna* leaves its summer residence to hibernate, *microgyna* stays all winter merely going deeper into the soil in cold weather (and thereby resembling *M. scabrinodis*); and that the sub-colonies produced by budding in *microgyna* appear nevertheless to have very little social contact, for dyes introduced do not spread. Further differences consequent upon these will appear in the course of this paper.

Throughout this paper it is proposed to symbolise *macrogyna* as M and *microgyna* as *m*. Also, the word "females" is used to include all members of the female sex: workers, virgins, and queens. The last two of these, collectively "sexual females," are distinguished on a basis of whether they have (queens) or have not (virgins) copulated and removed their wings.

These forms were originally described as varieties, but following a decision of the International Commission on Zoological Nomenclature at Paris in 1948 they have been automatically elevated to the rank of subspecies. There are, as will later appear, good reasons for elevating them still further but until more information about their geographical distribution is available this step is considered premature. They exist side by side in the mid-west Scottish area in which they were discovered and first studied, and intermediates in form, size and behaviour which are probably hybrids exist but are uncommon.

Normally an early step in the analysis of such a situation would be to perform hybridisation tests but this cannot yet be done with ants for two principal reasons: first, that copulation cannot be induced in the laboratory in all species (including this one) as fairly precise climatic, physiological and psychological conditions appear necessary (as in *Apis mellifera*); and secondly, that even if this were attained the females would then need to produce mature colonies before they could yield daughter females, and this has only recently been accomplished in ants under laboratory conditions.

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[2] Personal communication from J. Weir.

TABLE 1. *The distribution of head-widths of copulating pairs*

		Head-widths females (x)										
		31	32	33	34	35	36	37	38	39	40	Sum
	36	—	—	—	—	—	—	—	—	1	—	1
	35	—	—	1	1	1	2	1	7	1	2	16
	34	—	2	5	2	—	4	2	8	7	1	31
	33	2	—	2	2	1	—	6	14	4	4	35
Head-widths	32	1	3	6	4	1	1	6	7	5	1	35
males (y)	31	1	5	2	5	—	2	10	5	—	—	30
	30	2	1	8	2	2	2	1	2	—	1	21
	29	2	5	2	3	—	1	—	1	1	1	16
	28	—	—	1	2	—	—	—	—	—	—	3
	27	—	1	—	—	—	—	—	—	—	—	1
Sum		8	17	27	21	5	12	26	44	19	10	189

The regression equation $y = 0.292x + 21.6$ gives the best fitting straight line. One unit of head-width = 0.03026 mm.

For these reasons, recourse has been made to indirect methods.

NUPTIAL FLIGHTS

The Stations Studied

This species of ant like other *Myrmica* species (but not ants in general) copulates on the ground in what will be termed a mating station. For their generation these areas appear to necessitate the conjunction of a vertically elevated marker (tree, house, etc.) and a bare, roughly plane surface below or near. Thus paths beside trees or houses are often used. Males come to these stations and fly in groups settling on the ground or other surface or running actively about, and when they meet a female (sexual or worker and not necessarily of the same species) they attempt copulation without ceremony. Frequently the sexual female does not cooperate.

Such flights occur after the females have matured (in late July) on several climatically suitable days during August or early September. The males, which predominate, persist throughout the period (seeking refuge between flights in cracks and under vegetation) but the females come, copulate and leave. Intraspecies crossing is assisted by the choice of a similar and restricted site for mating and by the nearly synchronous emergence that takes place in the late afternoon under special climatic conditions: no wind, falling temperature after a warm day, no rain and usually little cloud.

The populations of three stations have been specially studied:

(1) the roof of a caravan surrounded by rough grazing on a rocky knoll;
(2) a gravel cart-track passing through a strip of regenerating moorland;
(3) a similar track passing through a belt of partly felled pines, and rough grazing.

Near all these, mixed populations were known to occur, as well as the other species *M. scabrinodis*.[3]

These stations were visited whenever mating flights were expected, and copulating couples collected together with a sample of the male population present. Later, as an index of individual size, the width of the head across the eyes was measured (as described in *loc. cit.* 1949); the units used were also identical. In future in this paper reference to individual "size" means to head-width.

[3] This species uses the same mating grounds as *M. rubra* but tends to mate rather later in the season.

Copulating Pairs

In collecting samples, careful attention was paid to the degree of association between the pair, and it was only when full genital contact existed that a pair was captured. From the three stations 189 couples were collected in all, and the distribution of their head-width pairs is shown in table 1.

The correlation coefficient between these two variates is $+0.418$ ($P < 0.001$) so that on the whole like size matings preponderate and some mechanism must exist favouring this. Some idea of the degree of cross-mating may be obtained by studying the extreme sizes, which can be allocated with fairly high probability to one or other sub-species. It was shown by tests described in the earlier paper (*loc. cit.* 1949) that queens of size 36 and over were M for the most part, and queens of 34 and less were *m*. Thus omitting the central class (35 units) the female population can be divided into two parts. A division of the male population is less easy as they, after being classed through the queens in their nest of origin, overlapped in size so much as to form a unimodal platykurtic distribution. However, males of 33 units or over are M with probability 97% and those of 29 units or less are *m* with a probability of 92% (table 13, *loc. cit.* 1949).

Hence the four "corners" of this mating table (Table 1) represent nearly pure like- and cross-matings respectively. This shows 16 intra-*m* and 58 intra-M matings, 17 *m* female × M male and only 4 M female × *m* male matings. Thus the mechanism favouring inbreeding probably operates more strictly against crosses of the last type involving M females and *m* males. An explanation of this is suggested later.

Autoselection

The most obvious cause of such a correlation between the sizes of pairs *in copula* is a mating preference and since males are very catholic in their choice of objects for approach, this must, if it exists, lie with the female. To investigate this aspect, the sizes of males copulating and not copulating were compared on any particular mating occasion (for clearly pooling data from several places and several occasions would be expected to obscure rather than clarify any relationship). Only six sets of data provided sufficient material for analysis by the χ^2 method of comparing frequency distributions, and in none of these was any significant difference obtained.[4] Hence if queens do have preferences they are feebly manifested.

An indirect approach to this problem was tried. Workers were tested to see if they showed signs of discriminating between the two extreme male sizes. This was done in the same way as earlier (*loc. cit.*, pp., 400–2) the worker reaction to *queens* of various sizes had been tested by setting up 3 laboratory cultures of each species and running in *males* of known size. No evidence of discrimination was obtained, all the males being treated with mild hostility. On the other hand, the workers recognised males of *scabrinodis* and reacted violently.

Thus if workers cannot distinguish between males (as they can between females) it is likely that queens cannot either, which supports the conclusions derived from the mating measurements. Other causes of the positive correlation between couple-sizes must be sought.

Nuptial Composition

(a) *Spatial variation:* The frequency distributions of sizes of all females and males collected at the three mating stations on different nuptial days in 1948 have been tabulated (table 2). From this it appears that both sexes show a smaller average size at station 2 than at the others, which in fact resemble each other. Thus on 31 July the mean sizes for stations 1, 2 and 3 respectively were: (males) 33.2, 31.4, 32.9; (females) 37.6, 33.8, 38.7. This spatial

[4] In the case with most difference between the two types of male a probability of between 0.10 and 0.05 was calculated.

TABLE 2. *Frequency distribution of the head-widths of all queens and males collected at the mating stations*

Sex	Station	Date	Head-width[1] 26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	Average
Males	1	27 July	—	—	—	1	—	2	9	17	7	8	1	—	—	—	—	33.2
		31 July	—	—	—	—	—	5	10	10	13	7	—	—	—	—	—	33.2
		13 August	—	—	—	5	12	19	15	18	16	5	—	—	—	—	—	32.1
		27 August	—	2	4	7	9	20	15	20	9	2	—	—	—	—	—	31.5
	2	31 July	—	—	5	3	6	6	3	7	6	2	—	—	—	—	—	31.4
		13 August	1	1	9	28	27	26	22	10	5	—	—	—	—	—	—	30.5
	3	31 July	—	—	—	1	1	—	2	6	5	1	—	—	—	—	—	32.9
		27 August	—	—	1	3	11	11	46	36	21	12	5	—	—	—	—	32.6
		28 August	—	—	—	1	4	17	19	22	16	14	—	—	—	—	—	32.7
		29 August	—	1	1	1	11	15	17	18	8	4	—	—	—	—	—	32.0
Females	1	27 July	—	—	—	—	—	—	—	—	—	1	1	2	7	5	4	38.3
		31 July	—	—	—	—	—	—	—	1	2	—	2	—	7	8	—	37.6
		13 August	—	—	—	—	—	2	2	10	5	1	1	11	13	—	—	35.5
		27 August	—	—	—	—	—	1	1	5	3	—	2	10	15	3	—	36.5
	2	31 July	—	—	—	—	—	1	3	3	3	1	1	—	—	—	1	33.8
		13 August	—	—	—	—	—	4	11	8	5	1	2	—	—	—	—	32.8
	3	31 July	—	—	—	—	—	—	—	—	—	—	1	2	3	5	5	38.7
		27 August	—	—	—	—	—	2	3	6	6	1	22	24	29	13	4	36.8
		29 August	—	—	—	—	—	—	3	2	—	3	3	10	9	2	—	36.4

[1] One unit of head-width = 0.03026 mm.

difference is due to the fact that the sexual forms do not travel far from their nest to a mating station. They appear to be drawn to the nearest; hence local population differences, caused as will be shown, by ecological differences, are reflected in the composition of the nuptial population.

A second spatial factor favouring segregation is the fact that *m* often mate just outside their nest (on tree stumps for example) and do not go to the mating grounds at all, a behaviour feature that has not been noticed with M.

(b) *Seasonal trend:* It will be noticed that at all three stations and in both sexes there is a tendency (with minor deviations) for the mean size to decrease as the season progresses. It is quite clear that in stations 1 and 3 the population is initially almost pure M (size 36 units and over) and only later becomes mixed. But in no case, even amongst females, is a state of pure *m* reached. This trend can be detected even within the smaller size groups which are presumably mostly *m*. Thus queens were caught whilst seeking nests on tree stumps shortly after mating [5] on nine occasions at station 2 (table 3). Even though predominantly *m*, they showed an over-all reduction in size during the period 5–29 August.

These temporal shifts in population structure may result, as will appear after ecological distribution has been dealt with, from the occupation of warmer sites by M; sites that are for a few years very much better than the more durable sites occupied by *m*. Comparison of nests earlier in the season showed that in those of M the virgins were much more advanced than in those of *m*: they were all emerged and fully pigmented adults instead of being still in part pupal and (if emerged) pale.

(c) *Annual variation:* No collections

[5] Once 18 were found on a single stump.

were made at mating stations in other years but a large number of nests were always examined before the nuptials and it was found that while M virgins were always produced in suitable nests though the numbers seemed to vary from year to year, in *m* nests in many years sexual brood could not be found, and only in 1948 (by good fortune the year of the mating study) of the five years 1948–53 of continuous record, were *m* virgins really common.

This might be a climate-induced phenomenon, for the August of 1947 was unusually fine and warm and this is the period when sexual brood is in its early larval stages. On the other hand, there are theoretical grounds for supposing that intrinsic cyclical variations in sexual production might occur in *m*, the co-phasicity of the various colonies being maintained by just such climatic aberrations. These cycles are likely to result from secondary pleometrosis if the queen intake after a good year is so great as to create in the following spring an excess of eggs in relation to workers, and, as a consequence, undernourishment of the brood and worker-formation. Several years might be needed before the worker population could be brought to a level where sufficient service would be available for the young brood to convert it into sexual forms. Such a cycle would of course be avoided if the workers controlled the queen intake of their colony, and then, as with M, sexual production would occur each year (once maturity was reached).

Thus this periodic sexual production on the part of one of the subspecies would mean that in many years cross-matings were very infrequent, and it would therefore add to the tendency already noted (Table 1) in the good *m* year 1948, in favour of inbreeding in the M population.

Conclusions

The positive correlation in size between pairs *in copula* observed during a year when sexuals of both forms were abundant must be attributed not to mate preferences, but to spatial and temporal differences in distribution. This tendency to inbreeding is likely to be increased in other years since *m* only produces sexuals sporadically. Moreover *m* tends to mate near the nest without flying to the mating grounds.

The fact that little pairing of M females with *m* males occurs even amongst those that in good years reach the mating stations, is probably due to the fact that the smallest *m* males only appear late in the season after most of the M females have mated and parted.

TABLE 3. *Frequency distribution of the head-widths of 116 deälate queens found on the ground at mating station 2*

Date	Head-width*									Sum	Average
	29	30	31	32	33	34	35	36	37		
August 5	—	—	—	2	2	3	2	3	2	14	34.6
August 6	—	—	1	2	5	1	2	2	1	14	33.8
August 8	—	—	—	—	1	2	1	2	—	6	34.3
August 11	—	—	1	5	4	4	6	3	—	23	33.8
August 20	—	—	2	3	—	1	—	—	1	7	32.7
August 24	1	3	8	8	1	1	—	1	—	23	31.6
August 26	—	—	2	2	3	2	1	—	1	11	33.2
August 28	—	3	1	2	2	—	—	—	—	8	31.5
August 29	—	1	2	4	3	—	—	—	—	10	31.9
Sum	1	7	17	28	21	14	12	11	5		

* One unit head-width = .03026 mm.

TABLE 4. *The distribution of head-width among the colonies of* M. rubra *sampled from two local areas in the 1949 survey*

Locality	Head-width[1]												Sum
	29	30	31	32	33	34	35	36	37	38	39	40	
Lady's Lees	—	5	8	14	7	4	4	21	14	4	1	—	82
Feddan's Wood	2	4	7	18	15	10	5	16	39	31	12	2	161

[1] One head-width = .03026 mm.

LOCAL DISTRIBUTION

General

The differences in colony reproductive method that these two subspecies show would be expected to lead to a difference in type of habitat occupied. M, with mortal colonies of wide dispersal power, and the ability to shift bodily every winter, would be expected in vegetational seres wherever these appeared. On the other hand *m* with theoretically immortal colonies, but low dispersal power would be expected in climax vegetation provided this allowed solar warmth to reach near enough to the soil (Brian and Brian, 1951).

The conditions under which they can enter a habitat are not necessarily those under which they can survive. Thus for M, entry must usually be through solitary foundress queens which, being unable to build mounds in herbs and grass, are probably [6] only successful if they can find bare or at most lichen-and-moss-covered ground in which to excavate. Once workers are plentiful, the shading action of an herb layer can be overcome to a limited degree by mound construction, and this subspecies can exist in the same habitat type as *m*. This latter subspecies, having no single foundress queen stage in its life-history is independent of these entry conditions. As a modifier to these general conclusions it should be added first that M colonies are able to move *en masse* and secondly that bare areas often appear in herb strata—caused by animal action, and the movement of stones.

With the aid of these principles, it was found that certain curious distributions could, after considerable research into the history of the site, be explained. But before detailing these it is convenient to list the habitat types in which one or the other occurred alone. M was the sole form found in a felled-woodland subsere (described in Brian, 1952), in a plantation of pines and in burn subsere of heather (both described in Brian and Brian, 1951). Presumably its queens had penetrated into each when bare soil (for nest-sites) was interspersed with herbs (for food) and the colonies had matured quickly enough to "fruit" before obliteration by the closing canopy. The other subspecies, *m*, was found almost alone in many places on the south-facing margins of agricultural fields, banks, hedgerows, walls, etc., and in grass especially where stones had lain about long enough in one position to give a chance of settled conditions. Most of these field boundaries have existed unchanged for at least a century as examination of the map of the Ordnance Survey for 1860 shows; indeed, since that time there has been a reduction in the number of boundaries rather than an increase. Most probably in the area studied they were constructed in the second half of the eighteenth century when the estate in question (Colgrain) was reclaimed.[7] Stable sites other than boundaries before that

[6] Sufficient experimental results have not yet been obtained to enable a firmer statement to be made.

[7] The then owner (of the family of Dennistoun) obtained a medal from the Highland Society in 1801 in respect of "improvements"; and the march dyke on the east side was built in 1766.

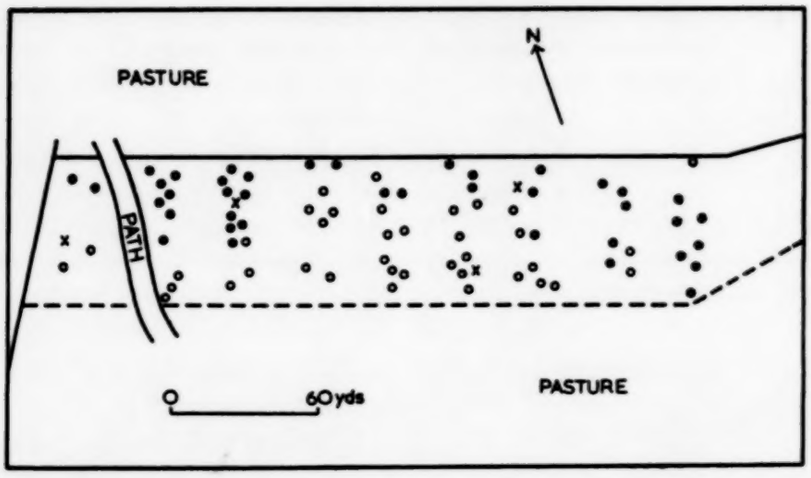

FIG. 1. Lady's Lees: a map of the distribution of the forms of *Myrmica rubra* L.; black circles, sexual females of head-width 36 divisions and over (principally *macrogyna*); white circles, size 34 and less (principally *microgyna*); crosses, size 35 (intermediate). The northern boundary is a fence, the southern a ruined wall.

period would most probably have lain around farm buildings, sheep-folds, trackways, etc.

The two species occurred together in places where a sere had been deflected into biotic climax grassland: either heather-moor after burning and sheep stocking or woodland after felling and invasion by farm stock. Thus in general distributional studies confirm that the reproductive differences between the forms adapt them to seral and biotic plagioclimax vegetation types respectively. There has been no suggestion that edaphic, microclimatic, or special vegetational factors operate differentially.

Two Special Cases

Two areas where both subspecies occurred together were surveyed specially. Both were long, narrow strips of felled contour woodland, and they were surveyed by taking belt transects every 30 yards across the strip. This was done in July before nuptial flights so that sexuals were present in the nests. The first sexual female seen was taken and its head-width later measured in the laboratory. Thus the assessment of each nest was based on a single specimen, but in view of the correlations shown to exist between queen and progeny, and of the slight variance normally encountered (*loc. cit.* 1949), this method, which had the advantage of enabling more extensive sampling, was considered satisfactory.

In the figures (1 and 2) all records of sizes 36 and over have been indicated as M, all of 34 and less as *m* and all of 35 as indeterminate. The justification for this has been explained earlier in this paper, and also in the previous publication (*loc. cit.* 1949).

(a) *Lady's Lees:* This area is a belt of Pine woodland (fig. 1) of area 3.2 acres that has been so thinned that very few trees (all mature however) have been left standing. It is on a slope with southern aspect, and at present cattle graze the grass, entering from below over the remains of a stone wall; the top side is fenced. Ants (principally *M. rubra*) nest in the old tree stumps and under stones. The figure clearly shows a disposition of *m* centrally and near the lower (southern) edge, M forming a complementary distribution along the top and at the two ends.

The history of this area obtained from maps and estate documents[8] shows that it was planted before 1835 and felled

[8] It is a pleasure to acknowledge the courtesy of Mr. Colin Campbell who gave us access to these.

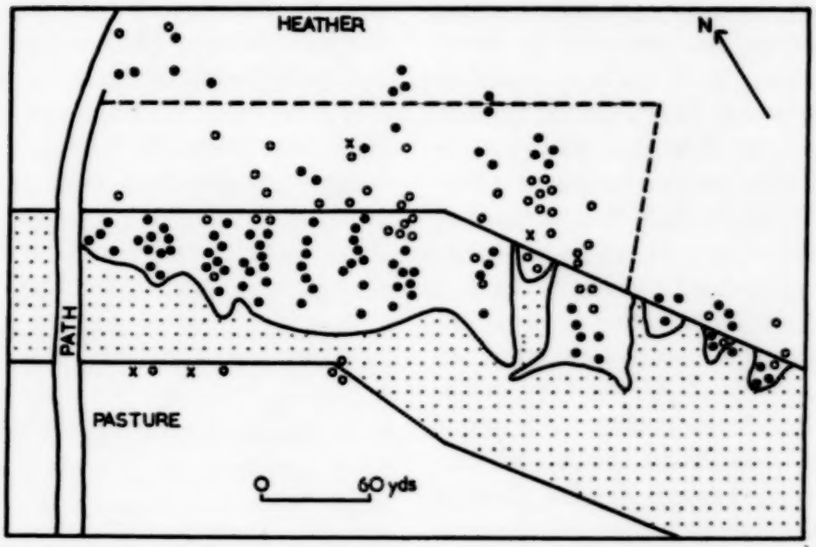

FIG. 2. Feddan's Wood: a map of the distribution of the forms of *Myrmica rubra* L.; black circles, sexual females of head-width 36 divisions and over (principally *macrogyna*); white circles, size 34 and less (principally *microgyna*); crosses, size 35 (intermediate). The southern part (dotted) is dense Pteridietum. Above the wood, the site of an old croft is marked (broken line). Both margins of the wood are bounded by fences.

sporadically from 1920 onwards. The wall on the south side was evidently ruinous before 1860 as it was not marked on that edition of the Ordnance Survey map, and today its traces remain as a grass-covered ridge with only occasional stones. It is along this sunny side of the old wall and under the stones that remained after its disintegration (and partial removal) that for a full century or more an ideal site for *m* probably existed. Except perhaps for a few small M colonies, no ants would have lived in the wood, for dense tall conifers exclude them (Brian and Brian, 1951). As the trees were slowly removed from 1920 onwards, giving heat and light at the soil surface, and providing rotting tree stumps, *m* would be able to travel upwards, and M to expand if present, and also to enter. This seems the only explanation that satisfactorily accounts for such a curious partition of habitat.

Mating station 2, which has been mentioned as being rich in *m*, was on the path going through the west end of this wood, and no doubt many of the sexuals from this population resorted to it, but some of the *m* unquestionably mated on the tree stumps (as already mentioned).

(b) *Feddan's Wood:* This wood of 22 acres, shaped as in the diagram (fig. 2), lies on a south facing slope and was, in 1949, in a state of subseral regeneration after felling in 1937, twelve years previously. Much of it is occupied by bracken (*Pteridium aquilinum*) growing so densely as to eliminate ants; the rest is a mixture of birch scrubland interspersed with acidic grassland sporadically grazed by sheep and rabbits.

The figure shows that a (perhaps pure) *m* population occupies the south boundary adjoining a meadow, nesting under stones on a small bank of material thrown out in the construction of a ditch. This boundary had existed for at least a century. No doubt any tendency to spread up into the wood after felling, as probably happened in Lady's Lees, was stopped by the rich growth of bracken, itself luxuriating in the increased light consequent on felling.

But the scrub grassland of the subseral area was almost wholly occupied by M nesting in the stumps and making mound nests in between these; presumably they had entered shortly after felling. Less easy to explain was the irregular band of *m* along the top (north boundary) of the

wood adjoining acidic grassland with patches of heather, for there is no trace of a wall or dyke, and such a situation would have been too shady in all probability (on the north side of a mixed pine-oak wood) to have enabled ants to exist. Early maps showed that this boundary had existed for at least a century and that the adjoining grassland to the north had been a croft, although no trace now remains except some odd rectangles of heath vegetation in otherwise pure grassland (where buildings had stood) and a spring that was obviously the water supply. This croft, known in its day as North Colgrain, was destroyed and its stones used to build a byre and a wall nearby, about the year 1880; but during its existence it must have provided a number of stable areas suitable for *m* colonisation, at least near to the wood margin.

In addition to the croft, an old road was discovered, through the existence of sporadically but linearly distributed stones, which ran very near to the wood margin for most of its course. Excavation showed that this was largely overgrown by soil and vegetation to a depth of 3 inches, and the presence of trees 120 years old in its centre showed that it had long been out of use: in fact it was not marked on the edition of the Ordnance Survey map of 1860. But its structure, especially the use of stone culverts over watercourses, indicated that it was built in the eighteenth century probably by the same Dennistoun that was awarded a medal for "improvements." This road with its edges and clearing may have provided a suitable *m* site for perhaps 2 centuries and if it deteriorated towards the end of this period the ant population would not have had to migrate far to reach the croft margins.

The sporadic M in the acidic grassland above the wood may have entered in the seral stages following demolition of the croft: for it was known by local people that arable land had existed there.

Thus again, although this case is more complicated, investigation has made it possible to account logically for the observed distribution of these two subspecies. Mating station 3 was situated on the path through the west of this area; and west of the path again was an area of almost pure M. It is not surprising that this station was rich in this form.

A Local Population Difference in M

The frequency distributions of head-widths obtained in these two surveys are given in table 4. Attention is drawn to the figures for the sizes 35 and over, that is to the predominantly M group. It will be noticed that those from Feddan's Wood were larger (χ^2 test, $P < 0.001$) than those from Lady's Lees. Such differences between local species populations are well known in less mobile species, but as these areas are only about 250 m. apart the difference is surprising. It is possible of course that it is purely phenotypic; Feddan's Wood was certainly in its optimal stage of development at the time of the survey, whereas Lady's Lees was grazed by cattle—the least desirable of all grass-cutting animals from the ant point of view, since their heavy hooves destroy ant nests as well as causing the death of numerous foragers. Phenotypic variations in head-width in this species have been described (Brian and Brian, 1951).

The difference in the *m* populations that the data suggest is not statistically significant (P: 0.5–0.7).

Discussion

In the discussion in the earlier paper on this subject (*loc. cit.* 1949), it was pointed out that species subdivided into two forms like those of *M. rubra* might not be uncommon in ants. Since then it appears from the evidence of Ledoux (1949) and Way (1954) that this may apply to another: *Oecophylla longinoda* Latr. At that time, the way was left open for the possibility that these forms were polymorphic manifestations of a fully interbreeding species-population, which whilst continually yielding individuals intermediate between the modes, never suc-

ceeded in establishing them owing to their lack of reproductive specialisation compared with the extremes. However, it is clear that this is only an early stage in an inevitable speciation, caused by the development of spatial and temporal separation under ecological influences such as have been shown to exist in this study. Although the bulk of the evidence is thus in favour of regarding these two forms as species, it is thought that until more is known about the geographical distribution it is premature to so elevate them, and the subspecific rank has therefore been maintained.

It is likely that the fundamental dichotomy of the species arose through the evolution of two reproductive methods specialised for seral and biotic plagioclimax vegetations respectively. This must have led immediately to spatial separation through the discontinuous distribution of those habitat types as well as through the tendency of *m* forms to mate away from the popular mating stations. From this spatial separation consequent temporal separations would develop—such as the earlier emergence of M through living in warmer habitats. In addition, as a direct consequence of the reproductive dichotomy a further temporal differentiator—the difference in the annual frequency of sexual production—would be superimposed.

The closest species to *M. rubra* today is *M. laevinodis* Nyl., a form whose specific validity is still disputed by some authors. As was pointed out earlier (*loc cit.* 1949) this species is not polymorphic,[9] it has queens as large as M queens, and yet its colony reproduction utilises both the methods seen in *M. rubra.* It is in fact less specialised in this sense in relation to habitat potenialities, and it may be supposed that each queen either returns to her nest, enters another nest, or founds a new colony alone or in the company of other queens, according to circumstances —which means of course that in dense old established populations secondary pleometrosis will be the rule.

It is easy to envisage if such a population could endure for long enough a loss of queen-size and adaptability leading to an *m* type, for under such circumstances there could be no selection pressure favouring larger size, and spatial isolation could be achieved in the absence of seral habitats or if these were occupied by other species capable of preventing the maturation of any colonies that succeeded in gaining a foothold in the early stages.

Conversely, the evolution of a dispersal-specialist type (like M) could perhaps take place from the general *laevinodis* position in a persistently turbulent habitat. On remeeting, no additional isolating mechanisms need have been evolved, for the various spatial and temporal factors discussed might, by themselves, have been adequate to retain the two individualities.

If *M. laevinodis* were the phyletic ancestor of *M. rubra,* its present patchy distribution in this country might be explained as a case of the replacement of a less adapted species by a more adapted specialist pair.

It does not seem necessary that the spatial isolation facilitating speciation should have been on a geographical scale, even though the condition of the ecosystem prior to human influence was different as palynology has shown. Today the seral habitats inhabited by M are all man-initiated subseres with the single exception of the moss-centres of old *Calluna vulgaris* plants (Brian and Brian, 1951). But this cyclic mosaic type of vegetation may have existed in mature natural forests where trees died or were blown down; and burn subseres may not have been unknown. M might also have extended into the tundra near retreating glaciers if the climate was sunny enough, as well as into the high screes and other shifting areas of erosion. Permanent habitats suitable

[9] Very recently, it has been pointed out to us by M. Bibikoff and by C. A. Collingwood that, in some European localities, microgynes of *M. laevinodis* occur frequently and a division of the species on similar lines to that of *M. rubra* may exist.

for *m* could probably be found as small areas of biotic climax grassland in forests maintained by the grazing of various ungulates such as the auroch, deer and bison. Although M might enter at the start of the sere that preceded the climax they would be likely to be entirely replaced by *m* owing to their comparative ephemerality and vulnerability as colonies, and their probable inability to enter once herbs had developed.

Summary

The degree of reproductive contact between the populations of two intergrading forms of *Myrmica rubra* L. (now designated as the subspecies *macrogyna* and *microgyna*) is considered.

Like matings are more frequent than unlike, but no good evidence of selection by the individuals concerned could be obtained. Rather it appears to be due to circumstances created by differences in the space-time distribution of the populations.

Such differences arise directly and indirectly from the reproductive differences between the subspecies which adapt them severally to seral (*macrogyna*) and biotic plagioclimax (*microgyna*) vegetation respectively.

Such intermediates (assumed to be hybrids) as occur are likely to be subviable for socio-ecological reasons (although physiological and genetic ones may reinforce these).

It is suggested that evolution from a less specialised form must have occurred during spatial isolation under different ecological conditions.

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MAN, POLYPLOIDY AND FRUIT TREE GROWING IN BRITAIN [1]

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INTRODUCTION

Genecologists have mainly concerned themselves with wild plant species occurring in natural floras, or in those disturbed by man. So far there has been little attempt to study the evolutionary ecology, in the broadest sense of the term, of cultivated plant species, more particularly of those varieties of fruits grown in orchards. In Britain new growers tend to confine their choice of fruit varieties to those already locally growing and cropping satisfactorily; they are also somewhat restricted to varieties suiting nearby markets. Yet these practices might themselves be influenced ultimately by the reactions of different varieties to local ecological conditions.

Man, of necessity, grows only those plants which he wants, either because they are profitable or because he likes them. Just as there are differences in food preferences between peoples of different countries, so there may also be local differences in taste preferences between people in the north and in the south of the British Isles. Such a factor would influence, for example, whether sweet or sour cherries, or whether dessert or culinary apples are in local demand. There could also be geographical differences in the domestic uses of fruits, which would be reflected in the requirements of different markets. Thus probably more fruit-pies are eaten in mining and heavy industry areas (i.e. northern counties of England), whereas more dessert fresh fruit is eaten in areas of commerce and light industries (i.e., southern English counties). Furthermore, historical factors in horticultural practices of different regions will no doubt have played a role, so that orchards in the north and in the south of England may have evolved in different ways.

In the hope of determining some of the basic factors *inter alia* which ultimately influence growers unconsciously in their preferences, it was decided to investigate the distribution of cultivated fruit-trees in Britain by using polyploidy as a biological yardstick, as previous investigations with polyploidy and wild species have proved profitable. The results besides having theoretical interest for evolutionists and geneticists might be of practical value to fruit growers. This paper, therefore, reports investigations on polyploidy in relation to the distribution of cultivated apples and cherries in Britain.

POLYPLOIDY, ECOLOGY AND GEOGRAPHICAL DISTRIBUTION

Various workers have investigated relationships between chromosomes and plant ecology since Gustafsson (1947) analysed the associations between life-form, vegetative reproduction and polyploidy. An account of the role of polyploidy in plant geography has been given by Cain (1944) and by Stebbins (1950), who emphasised that there is no unanimity as to the effects of polyploidy on distribution. A general summary of the role of chromosomes in plant ecology, and how it effects the distribution of wild species, especially in Great Britain, is given in Haskell (1954*a*).

SOURCE OF MATERIAL

Data were used from the Orchard Fruit Census, issued January 1951 by the Collection of Statistics Branch of the Ministry of Agriculture and Fisheries. This includes acreage and number of trees of

[1] Paper read originally to the Surrey Horticultural Progress Club.

dessert and cooking apples (excluding cider apples) both for trees under and over 7 years old. It also includes separate and similar data for sweet and sour cherries. The returns are for 47 counties in England, including separate entries for Isle of Ely, Soke of Peterborough and Isle of Wight; data for Soke of Peterborough and Northampton were pooled for analysis. There are returns for 13 Welsh counties, but data for cherry distribution in Wales are meagre, as many Welsh countries grow less than an acre. There are in Scotland only 300 acres of all varieties of apples, and few cherries; hence county analyses were not made for Scotland. Only data of trees seven years old and over are considered throughout this paper.

Data for preparing figure 1 were of apple trees in 59 countries; the original figures permitted only straightforward comparison between three diploid eating varieties for which separate returns are available, viz., Cox's Orange Pippin, Worcester Pearmain and Laxton's Superb, and one triploid culinary variety, Bramley's Seedling. Cox's Orange Pippin is the most commonly grown apple, with just under 4 million trees occupying almost 12 thousand acres in England and Wales. Bramley's Seedling is the second commonest variety, with just under 2 million trees occupying nearly 30 thousand acres in England and Wales. Comparisons were made between these two varieties.

FIG. 1. Areas showing relative distributions in English and Welsh counties of triploid culinary Bramley's Seedling apple trees, seven years old and over, compared with diploid dessert Cox's Orange Pippin trees, seven years old and over.

Black areas represent counties where the county ploidy ratios (*CPR*s) are above 4.1.

The situation with cherries is rather more complicated than for apples, as according to Crane and Brown's (1949) list about eighty sweet varieties are grown in Britain: they are grouped together in the Census. All sweet cherries are diploid (*Prunus avium*, $2n = 16$) of which there are just under 600 thousand trees occupying approximately 13 thousand acres. The tetraploid sour cherries (*P. cerasus*, $2n = 32$) comprise a few Morello varieties; there are 90 thousand sour cherry trees occupying a little over one thousand acres in England. These relative figures must be borne in mind when comparisons are made. The data for preparing figure 2 were for the number of cherry trees of all varieties in the 46 English counties only, as the total acreage in Wales is 24 acres of sweet and 4 acres of sour cherries.

THE COUNTY PLOIDY RATIOS

The ratios of polyploids to diploids are given county by county in table 1. They are the *County Ploidy Ratios* (*CPR*) and vary widely. For apples, Westmorland, Cornwall and the East Riding of Yorkshire have high *CPR*s while Northumberland and Carmarthen grow no Cox. At the other extreme, Bedford (the largest apple growing county) and Hertford grow few Bramley. For cherries, the highest *CPR* is that of Cheshire. Its value is much lower than the highest *CPR* for ap-

ples, i.e., 9.33 compared with 64.57. Essex, Middlesex and Leicester have comparatively high cherry *CPR*s, whereas, excluding Welsh counties, East Riding, Rutland, Worcester and Warwick, and many other counties, have very low *CPR*s. There are no diploid cherries grown in Westmorland and Kesteven (Lincs.), and none at all in Cumberland.

When apple and cherry *CPR*s were separately plotted on a county map considerable variation was found, even between *CPR*s of adjacent counties. For apples it was eventually decided arbitrarily to separate counties with $CPR = 0$ to 4.0 and above 4.1. In figure 1 blacked-in counties are those with *CPR*s above 4.1 and it is seen that higher *CPR*s preponderate in north-midland and northern counties (excepting Durham), in two Welsh counties and in Devon and Cornwall. Most of these counties have high ground and high rainfall, except those of the north-east coast. Counties with lower *CPR*s occupy the English Plain; they include the Home Counties and those of the south and east coast; low *CPR*s extend across the West Country into Wales.

The cherry data were similar, with wide variation from county to county, but having comparatively lower values compared with those of apples. Counties were grouped above and below 0.5 and in figure 2 blacked-out counties are those whose *CPR*s are above 0.5. The emerging pattern shows that high *CPR*s occur in counties of the north-midlands and northwest, and in Northumberland. There are also pockets of high *CPR* in Norfolk, Essex, Somerset and Dorset, and in East and West Sussex. Counties of the English Plain and along the north-east coast have lower *CPR*s, i.e. relatively more diploid sweet cherries are grown in these regions which have comparatively lower rainfalls. An evaluation must therefore, be made of those factors underlying regional differences between diploid and polyploid cultivated fruits.

FIG. 2. Areas showing relative distributions in English counties of tetraploid sour cherry trees, seven years old and over, compared with diploid sweet cherry trees, seven years old and over.

Black areas represent counties where the county ploidy ratios (*CPR*s) are above 0.5.

FACTORS AFFECTING DISTRIBUTION OF DIPLOID AND TRIPLOID CULTIVATED APPLES

1. *Economic characteristics:* Table 2, which compares economic plant and fruit characteristics, shows that Cox is liable to infection by apple canker on heavy and poorly drained soils and in wetter districts, whereas Bramley is resistant to canker but slightly susceptible to scab. Bramley is more suitable for growing on a wide range of soils and situations (cf. Ministry of Agriculture, 1950), and this may be one reason for higher *CPR*s in wetter areas of the north-west.

2. *Adaptation and heterozygosity:* It is possible with vegetatively propagated economic plants, like cultivated apples (often on standardized rootstocks), to maintain clones somewhat weaker through breeding

dessert and cooking apples (excluding cider apples) both for trees under and over 7 years old. It also includes separate and similar data for sweet and sour cherries. The returns are for 47 counties in England, including separate entries for Isle of Ely, Soke of Peterborough and Isle of Wight; data for Soke of Peterborough and Northampton were pooled for analysis. There are returns for 13 Welsh counties, but data for cherry distribution in Wales are meagre, as many Welsh countries grow less than an acre. There are in Scotland only 300 acres of all varieties of apples, and few cherries; hence county analyses were not made for Scotland. Only data of trees seven years old and over are considered throughout this paper.

Data for preparing figure 1 were of apple trees in 59 countries; the original figures permitted only straightforward comparison between three diploid eating varieties for which separate returns are available, viz., Cox's Orange Pippin, Worcester Pearmain and Laxton's Superb, and one triploid culinary variety, Bramley's Seedling. Cox's Orange Pippin is the most commonly grown apple, with just under 4 million trees occupying almost 12 thousand acres in England and Wales. Bramley's Seedling is the second commonest variety, with just under 2 million trees occupying nearly 30 thousand acres in England and Wales. Comparisons were made between these two varieties.

FIG. 1. Areas showing relative distributions in English and Welsh counties of triploid culinary Bramley's Seedling apple trees, seven years old and over, compared with diploid dessert Cox's Orange Pippin trees, seven years old and over.

Black areas represent counties where the county ploidy ratios (*CPR*s) are above 4.1.

The situation with cherries is rather more complicated than for apples, as according to Crane and Brown's (1949) list about eighty sweet varieties are grown in Britain: they are grouped together in the Census. All sweet cherries are diploid (*Prunus avium*, $2n = 16$) of which there are just under 600 thousand trees occupying approximately 13 thousand acres. The tetraploid sour cherries (*P. cerasus*, $2n = 32$) comprise a few Morello varieties; there are 90 thousand sour cherry trees occupying a little over one thousand acres in England. These relative figures must be borne in mind when comparisons are made. The data for preparing figure 2 were for the number of cherry trees of all varieties in the 46 English counties only, as the total acreage in Wales is 24 acres of sweet and 4 acres of sour cherries.

THE COUNTY PLOIDY RATIOS

The ratios of polyploids to diploids are given county by county in table 1. They are the *County Ploidy Ratios* (*CPR*) and vary widely. For apples, Westmorland, Cornwall and the East Riding of Yorkshire have high *CPR*s while Northumberland and Carmarthen grow no Cox. At the other extreme, Bedford (the largest apple growing county) and Hertford grow few Bramley. For cherries, the highest *CPR* is that of Cheshire. Its value is much lower than the highest *CPR* for ap-

ples, i.e., 9.33 compared with 64.57. Essex, Middlesex and Leicester have comparatively high cherry *CPR*s, whereas, excluding Welsh counties, East Riding, Rutland, Worcester and Warwick, and many other counties, have very low *CPR*s. There are no diploid cherries grown in Westmorland and Kesteven (Lincs.), and none at all in Cumberland.

When apple and cherry *CPR*s were separately plotted on a county map considerable variation was found, even between *CPR*s of adjacent counties. For apples it was eventually decided arbitrarily to separate counties with $CPR = 0$ to 4.0 and above 4.1. In figure 1 blacked-in counties are those with *CPR*s above 4.1 and it is seen that higher *CPR*s preponderate in north-midland and northern counties (excepting Durham), in two Welsh counties and in Devon and Cornwall. Most of these counties have high ground and high rainfall, except those of the north-east coast. Counties with lower *CPR*s occupy the English Plain; they include the Home Counties and those of the south and east coast; low *CPR*s extend across the West Country into Wales.

The cherry data were similar, with wide variation from county to county, but having comparatively lower values compared with those of apples. Counties were grouped above and below 0.5 and in figure 2 blacked-out counties are those whose *CPR*s are above 0.5. The emerging pattern shows that high *CPR*s occur in counties of the north-midlands and northwest, and in Northumberland. There are also pockets of high *CPR* in Norfolk, Essex, Somerset and Dorset, and in East and West Sussex. Counties of the English Plain and along the north-east coast have lower *CPR*s, i.e. relatively more diploid sweet cherries are grown in these regions which have comparatively lower rainfalls. An evaluation must therefore, be made of those factors underlying regional differences between diploid and polyploid cultivated fruits.

FIG. 2. Areas showing relative distributions in English counties of tetraploid sour cherry trees, seven years old and over, compared with diploid sweet cherry trees, seven years old and over.

Black areas represent counties where the county ploidy ratios (*CPR*s) are above 0.5

FACTORS AFFECTING DISTRIBUTION OF DIPLOID AND TRIPLOID CULTIVATED APPLES

1. *Economic characteristics:* Table 2, which compares economic plant and fruit characteristics, shows that Cox is liable to infection by apple canker on heavy and poorly drained soils and in wetter districts, whereas Bramley is resistant to canker but slightly susceptible to scab. Bramley is more suitable for growing on a wide range of soils and situations (cf. Ministry of Agriculture, 1950), and this may be one reason for higher *CPR*s in wetter areas of the north-west.

2. *Adaptation and heterozygosity:* It is possible with vegetatively propagated economic plants, like cultivated apples (often on standardized rootstocks), to maintain clones somewhat weaker through breeding

TABLE 1. *County ploidy ratios*

Figures are the ratios of polyploid to diploid trees (7 year old or over) of apples and cherries in England and Wales.

		County	Apples 3x Bramley / 2x Cox	Cherries 4x Sour / 2x Sweet
ENGLAND	1	Bedford	0.01	0.89
	2	Berkshire	1.10	0.04
	3	Buckinghamshire	2.14	0.09
	4	Cambridge	1.08	0.46
	5	Ely	5.75	1.52
	6	Cheshire	0.36	**9.33**
	7	Cornwall	**23.66**	0.15
	8	Cumberland	8.39	—
	9	Derby	4.46	0.78
	10	Devonshire	4.06	0.21
	11	Dorset	0.21	0.76
	12	Durham	2.69	0
	13	Essex	0.08	**2.64**
	14	Gloucester	2.09	0.06
	15	Hampshire	0.50	0.14
	16	Isle of Wight	0.12	0.42
	17	Herefordshire	1.52	0.01
	18	Hertfordshire	0.01	0.10
	19	Huntingdon	2.33	0.09
	20	Kent	1.60	0.12
	21	Lancashire	5.88	0.78
	22	Leicester	4.50	**2.00**
	23	Lincoln (Holland)	5.34	0
	24	Lincoln (Kesteven)	8.52	+
	25	Lincoln (Lindsey)	**16.71**	0
	26	Middlesex	0.80	**2.45**
	27	Norfolk	0.66	1.30
	28	Northampton	1.50	0.67
	29	Northumberland	+	1.17
	30	Nottingham	**11.89**	0.88
	31	Oxford	2.84	0.10
	32	Rutland	1.93	0
	33	Shropshire	1.07	0.22
	34	Somerset	1.12	0.58
	35	Staffordshire	**10.78**	0.63
	36	Suffolk (East)	0.82	0.43
	37	Suffolk (West)	0.28	0.42
	38	Surrey	0.65	0.38
	39	Sussex (East)	0.97	0.51
	40	Sussex (West)	0.35	0.60
	41	Warwick	0.53	0.01
	42	Westmorland	**64.57**	+
	43	Wiltshire	0.18	0.29
	44	Worcestershire	1.77	0.05
	45	Yorkshire (East Riding)	**22.88**	0
	46	Yorkshire (North Riding)	**15.14**	0.43
	47	Yorkshire (West Riding)	6.91	0.65
WALES	48	Anglesey	0.08	0
	49	Brecon	3.18	0
	50	Caernarvon	1.08	0
	51	Cardigan	0.72	0
	52	Carmarthen	+	0
	53	Denbigh	1.58	0
	54	Flint	0.19	1.00
	55	Glamorgan	0.72	+
	56	Merioneth	—	0
	57	Monmouth	2.20	0.12
	58	Montgomery	2.56	0
	59	Pembroke	0.73	0
	60	Radnor	**16.32**	0

+ means no diploids grown.
— means no figures available.

segregation and depression, which would be eliminated in wild populations. Hence some geographical differences between diploid Cox and triploid Bramley may be due simply to differences in genetical adaptation, irrespective of differences in ploidy. The greater vigour and better disease resistance of Bramley than Cox could therefore partly be a function of their relative heterozygosity; this acts alternatively or in addition to the direct effects of polyploidy.

3. *Frost resistance:* Both varieties are normally susceptible to frost but Bramley is more liable to damage (Bush, 1949) although both varieties flower at the same period. Bramley is one of the most susceptible of apples in spite of its blossom having a sturdy appearance. This might be related directly to polyploidy, as polyploids usually have larger cells than diploids, with increased water content and lower osmotic pressure. Hence polyploids may be more readily damaged by freezing (cf. Löve & Löve, 1949).

Climatic effects on fruit yield and quality may also have important consequences for the *CPR*s. Thus Cox either does not fruit under Scottish conditions, or if it does, its dessert qualities are markedly inferior to when it is produced in Kent or Essex (Dept. of Agric. for Scotland, 1949). No varieties recommended for growing in Scotland are early

TABLE 2. *Economic plant and fruit characters of Cox's Orange Pippin and Bramley's Seedling*

Character	COX'S ORANGE PIPPIN Diploid, $2n = 34$	BRAMBLEY'S SEEDING Triploid, $2n = 51$
Date Introduced	1850 (Middx.)	1876 (Notts.)
Growth	Weak grower with thin shoots and narrow rather pale leaves; not robust.	Exceptionally vigorous grower on all stocks. Large very dark leaves. Large spreading tree with exceptional constitution.
Climatic Requirements	On cold soils is not a profitable variety as develops canker; not dependable.	Somewhat susceptible to frost. Blossoms highly susceptible to frost damage; even unopened buds killed.
Soil Requirements	Requires good soil conditions and comparatively heavy manuring.	Succeeds well on all soils.
Disease Resistance	Subject to mildew and other diseases. Leaves very susceptible to attacks of scab. Foliage and fruits damaged by strong Cu and S sprays.	Fairly resistant to scab. Foliage not damaged by Cu and S sprays.
Fertility	Nearly self-sterile. Requires cross-pollination with other $2n$ varieties with similar flowering period.	Fairly self-fertile. Must be interplanted with one, preferably two, diploid varieties.
Cropping	Crops moderately if properly pollinated.	Slow to come into full bearing. Heavy cropper.
Fruit Characters	Finest eating variety of all English apples. Ready for eating in November. Keeps well until early next year.	Finest cooking variety. Ready November and keeps till March.
Marketable Properties	Readiest seller in all markets.	Once easy to sell; was in demand in the markets.

flowering (table 3) and this may be to avoid late frosts: there are also fewer late flowering varieties than recommended for England. Bramley is the only triploid of both dessert and cooking types especially recommended for Scotland, whereas in England Blenheim Orange (a dessert variety) and Bramley are the recommended triploids. Altogether in England and Scotland there are thirteen and ten dessert varieties respectively, and 9 and 1 culinary varieties.

4. *Self-fertility:* The relative self-fertilities of Cox and Bramley are shown in comparison with 48 other varieties in figure 3. Self-fertility is the fruit-set ex-

TABLE 3. *Flowering seasons and ploidy of cultivated apples recommended for growing in Scotland and England*

% Varieties

	Diploids				Triploids			
	Flowering			Total Diploids	Flowering			Total Triploids
	Early	Mid-Season	Late		Early	Mid-Season	Late	
Generally grown in Britain	**31.8**(27)	**50.6**(43)	**17.6**(15)	85	**41.7**(5)	**50.0**(6)	**8.3**(1)	12
Recommended for Scotland	**0** (0)	**90.0**(9)	**10.0**(1)	10	**0** (0)	**100** (1)	**0** (0)	1
Recommended for England	**9.5**(2)	**71.4**(15)	**19.1**(4)	21	**1** (0)	**100** (2)	**0** (0)	2

Actual numbers are in brackets.

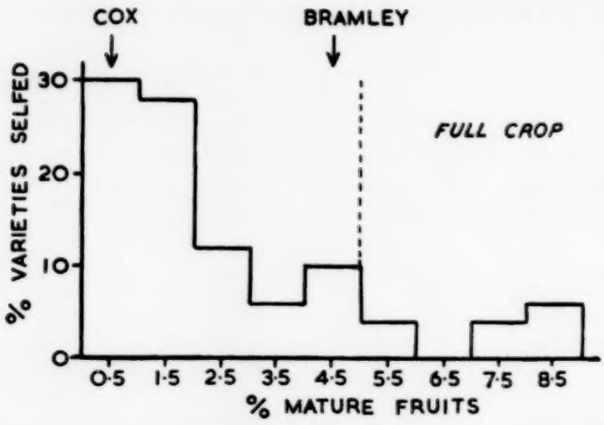

FIG. 3. The frequency distribution of fruit setting following self-pollination in fifty varieties of apples (Data from Hall and Crane, 1933). The relative self-fertilities of diploid Cox's Orange Pippin and triploid Bramley's Seedling are shown.

pected if trees of each variety were isolated from those of others, yet still available to insects. Cox sets very unsatisfactorily when selfed, but Bramley produces a 4.5% set, even though its pollen is not suitable for fertilizing diploid varieties. A 5% fruit-set is considered equal to a full crop; indeed, too many fruits on a tree leads to heavy dropping owing to nutrient and other limiting factors. Where bees are relatively few, as they might be in the north or in wet districts, a self-compatible variety like Bramley is at an advantage, for it may still produce a good crop. This might, therefore, affect the relative distributions of Cox and Bramley in England. Yet if bees are entirely absent, as in an insect-proof glasshouse, even a self-compatible variety like Bramley fails to fruit because apple flowers are not adapted to autonomous pollination. Thus although Bramley has the advantage of self-fertility, a full crop is set only with a sufficiency of bees for pollination. Hence self-fertility is not the only factor affecting geographical differences between diploid and triploid apples; it is further analysed later.

Now Butler (1945) has stated that at first sight it is difficult to see how cross-pollination is effected in an orchard, as individual bees have "fixation areas" on a small area of the crop. However, if there is a sufficiently large population of pollinators, as might occur in southern counties, there is a surplus of wandering bees which act as cross-pollinators. Related to these foraging habits of bees may be the relative density of Cox to Bramley throughout English and Welsh counties, viz., $\frac{4{,}000{,}000}{12{,}000}$: $\frac{2{,}000{,}000}{30{,}000} = 5:1$, and this, in turn, may be related to the practice of growing Bramley as orchard trees on vigorous stocks whereas Cox is often grown as cordons.

5. *Geological relationships:* Generally it appears that Bramley is grown to a less extent on areas where the underlying rocks are newer sedimentaries (new red sandstone), as found in the Cheshire and Shropshire Plains. On the other hand, more Bramley is grown where the underlying rock is Old Crystalline (palaeozoic) and also on alluvial soils around the Wash. However there may be disturbances owing to glacial drift. The exception of Durham may partly be explained by the artificiality of this county which is heavily built over and industrialized.

FACTORS AFFECTING DISTRIBUTION OF DIPLOID AND TETRAPLOID CULTIVATED CHERRIES

1. *Incompatibility:* Acid and duke cherries are only partially self-compatible, setting poor crops with their own pollen, but two varieties, "Morello" and "Kentish Red A" give a fruit-set of over 20%, which Crane (1938) considers satisfactory. What proportion these two varieties form of all tetraploid cherries in the Census is not known. Tetraploid morello cherries therefore set a good crop when self-pollinated, which means that when pollinating insects are scarce, chance self-pollination either within or between flowers on a tree still produces a satisfactory crop. Sweet cherries are not suitable for pollinating them. All diploid sweet cherries are self-incompatible; Crane & Brown

(1937) have found that self- and cross-incompatible pollinations give a fruit-set of 0.08%, whereas cross-compatible pollinations give 22.6%. There must be cross-pollination between compatible varieties flowering at the same time so growers have to arrange correct combinations in their orchards. Thus as with Bramley, the somewhat self-compatible sour cherries might be preferred in more northerly areas having greater vagaries of climate.

Comparison of *CPR*s for adjacent counties shows that self-fertility is not the only factor influencing choice. For example, Cheshire has the very high $CPR = 9.33$ compared with adjoining Shropshire with $CPR = 0.22$; Isle of Ely has $CPR = 1.52$ but it is 0 for Holland (Lincs.); the $CPR = 0.12$ in Kent, the county with most trees. Howes (1945) has pointed out that Kentish growers regularly place hives among trees at flowering time to ensure cross-pollination but in the north it may not be so easy to hire hives as cherry growing is not so concentrated as in Kent. One has to look further than fertility factors and pollination requirements to explain such local differences.

2. *Flower characters:* Sour cherries flower a little later than earliest flowering sweets, but well before the latest sweets, and duke cherries are latest of all but overlap with late sweet varieties (Beakbane *et al.*, 1935). These differences in flowering time between diploids and tetraploids are possibly of indirect assistance in avoiding frost damage to open flowers in which styles are killed. There are other differences between sweets and morellos, e.g., in the types of inflorescence, and morello flowers have lower stamen numbers than those of sweet cherries, with dukes intermediate (Haskell, 1954*b*).

3. *Bird damage:* Ingram (1948) has pointed out that isolated specimens of sweet cherry (*P. avium*) do not crop: even if they did he believed that birds would strip them before ripe fruits were harvested. Where bees are scarce, orchards of sweet cherries set poor crops owing to failure of cross-pollination and as birds are a major pest the crops are even further depleted. Observations of Mr. A. G. Brown at Merton suggest that birds may only strip sour cherries after finishing supplies of sweet. Birds are therefore a limiting factor in sweet cherry growing, especially for small-scale plantings, in contrast to apples.

TABLE 4. *Susceptibility to bacterial canker in 2x and 4x cherries*

	Extremely susceptible	Intermediate susceptibilities	Very slight susceptibility
2*x* Sweets	**21%** (22)	**46%** (48)	**33%** (34)
4*x* Dukes and Acids	**4%** (1)	**44%** (11)	**52%** (13)

Figures in brackets are actual number of trees.

(Data calculated from Grubb, 1937.)

4. *Tree hardiness:* There are differences in hardiness between diploid and tetraploid cherries: Ingram (1948) records that *P. avium* (*2n*) only grows satisfactorily in more southern and south-western districts of Sweden. *P. cerasus* (*4n*) is the hardiest fruiting cherry, and alone able to endure unharmed winters of central Sweden, where 50–60° frost can occur. Ingram also maintains that *P. cerasus* withstands a much damper climate than *P. avium* and it is noticeable that higher *CPR*s of cultivated varieties occur generally in areas where the mean rain days per annum is above 200. Hence physiological differences between diploid and tetraploid trees themselves may also be important in cherry tree distribution.

5. *Resistance to bacterial canker:* According to table 4, based on observations by Grubb (1937) at East Malling, there are considerably more sweet cherries than sour extremely susceptible to bacterial canker, which is often fatal. Final approval of a new sweet cherry bred at this Institution depends largely on its field resistance to bacterial canker. Montgomery & Moore (1945) have stated that high autumn rainfall favours the incidence

of canker in the following year; thus areas of the high *CPR*s may be indirectly related to the incidence of this disease.

6. *Geological relationships:* These are not so clear as for apples. It appears that tetraploids are grown to a less extent where the underlying rocks are newer sedimentaries.

Distribution of Wild Apples and Cherries

Warburg (1953) states that many fruit trees and shrubs have long been cultivated in Britain; some are undoubtedly native, although the wild form is not always the same. This applies to apple and sweet cherry; pears and medlars are undoubtedly introductions. Warburg also states that the sour cherry (*P. cerasus*) is perhaps grown less than formerly. Clapham, Tutin and Warburg (1952) claim that *P. avium* is native to British woods and hedges on the better soils, where it may be rather common. In contrast, *P. cerasus* is introduced; it grows in hedges and is local. They also believe that although the origin is uncertain, these wild plants are descended from the garden sour and morello cherry. According to Druce (1932), wild crab apple (*Pyrus malus* = *Malus pumila*), a diploid species with $2n = 34$, occurs throughout England, Wales and Ireland. In Scotland it occurs south of the line between Glasgow and Edinburgh (except for v. c. 82) and along eastern vice-counties; it is absent in parts of the north and north-west. The small acreage of cultivated apples in Scotland reflects the natural distribution of this wild species.

The comital distribution in Britain of the wild diploid *P. avium* and tetraploid *P. cerasus* (Druce, 1932) reveals that *P. avium* occurs throughout Great Britain, being absent only in a few vice-counties of northern Scotland. In Ireland *P. avium* is distributed in the north and along eastern counties, but absent from some central and western ones. *P. cerasus,* on the other hand, is restricted to English vice-counties south of a line between the Bristol Channel and the Wash, and in most of Wales. Reliable records in the north are only for north West Riding (v. c. 63), west North Riding (v. c. 65), Westmorland and Cumberland; it is particularly in this area of north-west England that *CPR*s of cultivated varieties are high (cf. fig. 2). Generally, then, tetraploid wild and cultivated cherries are considerably less common in the north-east than the north-west of England and this may be related to the differences in rainfall. *P. cerasus* is the more widely distributed in Ireland, being found in all vice-counties other than Kerry (H 1 and 2) and Clare (H 2). The wild tetraploid in Ireland seems more adapted generally to wetter and moister conditions, both confirming Ingram's (1948) observations and agreeing with what is known of the ecological preferences of polyploids (Haskell, 1951, 1952).

The wide distribution of *P. avium* throughout Britain, in contrast to limited distribution of *P. cerasus,* can be explained in several ways. Limitation due to glaciation is doubtful; more likely if both species are introduced, man has preferred to plant *P. avium* because of its upright growth and tree habit making excellent timber. Hutchinson (1950) says it is widely planted, but not *P. cerasus* which is a much branched bushy shrub with roots producing numerous suckers. The limited wild distribution of a self-compatible polyploid species in contrast to wider distribution of a self-incompatible related diploid species in nature, again confirms the view that man and other plant characters are more important than differences in fertility. This is now considered further.

An Evaluation of Self-Fertility

A map of diploid cultivated raspberry distribution in England and Scotland (Haskell, 1954*b*) shows that cultivated varieties are widely spread, with largest acreages in Perth and Angus. It has not been possible to determine the distribu-

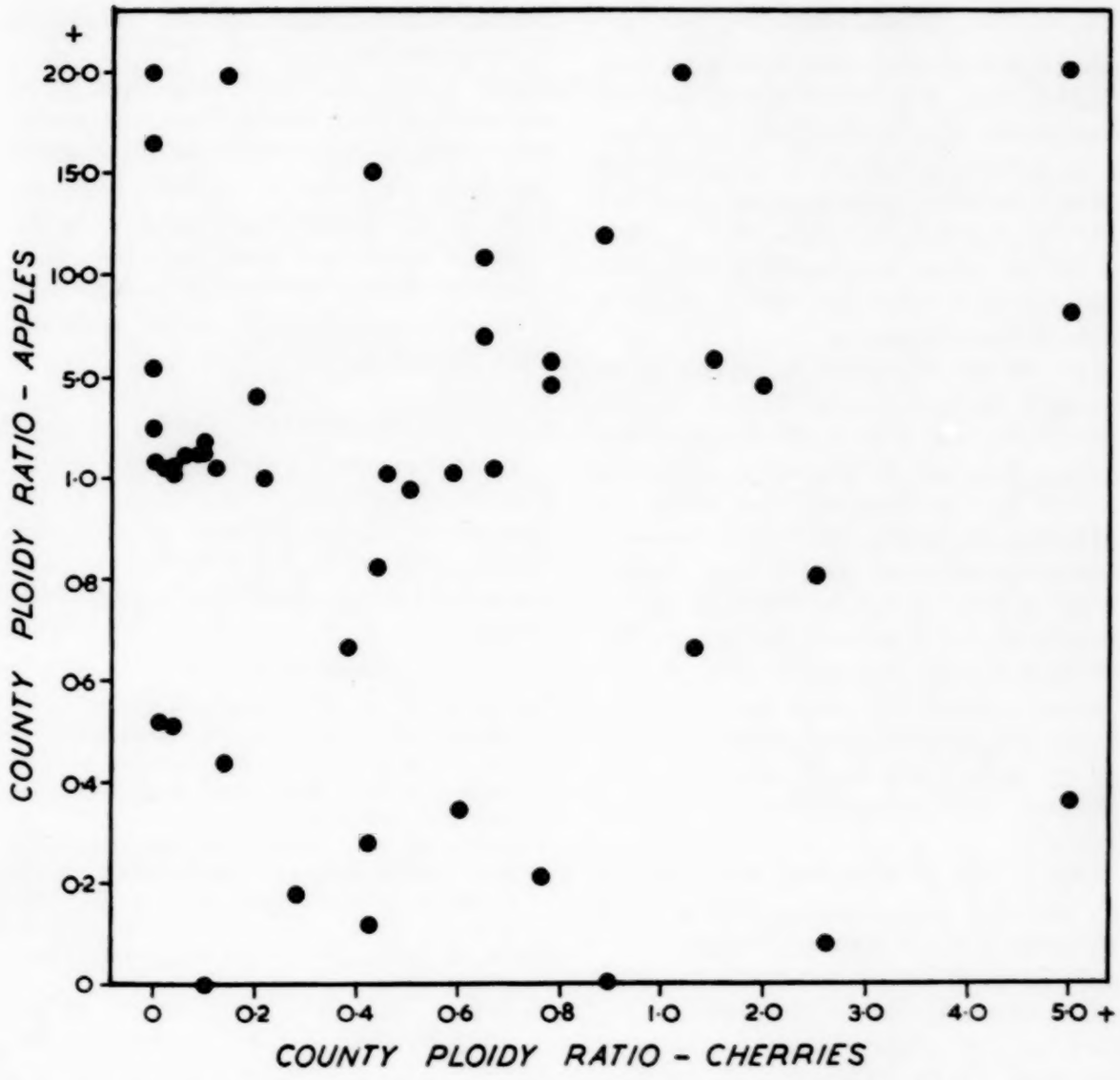

FIG. 4. County ploidy ratios (*CPR*s) for apples plotted against those for cherries. Data are for English countries only.

tion and abundance of polyploid varieties, but it is clear that the diploids are grown over most of Britain. As Crane (1948) has shown, raspberries are cross-pollinated even though they are self-compatible, probably because of pollen competition effects. This means that bees amply pollinate within the clone raspberry crops as far north as the large Scottish centres of the raspberry industry. Hence other local factors, such as type of soil and suitability to markets, are as important as genetical effects of adaptation and pollination requirements.

If self-fertility is a major influence on cultivated apple and cherry distribution, and so effects the *CPR*s, then the *CPR*s should increase as one goes northwards. Furthermore, the apple and cherry *CPR*s for each county should be correlated. However, no correlation nor regression is found when individual *CPR*s of apples are plotted against those for cherries as in figure 4. This may indicate that as the two groups of *CPR*s have no direct correlation, fertility factors, already known to play a role, are swamped by more important extraneous influences. What these are, and how they exert their effect, remain undetermined at present.

Summary

To help determine what influences man in his choice of fruit-tree varieties, the role of polyploidy in cultivated fruit-tree growing in Britain was studied for apples and cherries. County ploidy ratios (*CPR*s) were calculated as the ratios of the number of polyploid to diploid trees, seven years old and over, for each county of England and Wales.

The *CPR*s of triploid Bramley's seedling and diploid Cox's Orange Pippin were plotted on a map of England and Wales. Highest *CPR*s occur in north-midland and northern counties, in Devon and Cornwall, and in two Welsh counties; these areas generally have higher rainfall. Lower *CPR*s occur in counties of the English Plain. Factors influencing *CPR* distribution were evaluated: they include economics, plant and fruit characteristics, genetic adaptability, frost resistance, geology of underlying rocks, and self-compatibility. All are considered to have effects.

The *CPR*s of tetraploid sour cherries (*P. cerasus*) and diploid sweet varieties (*P. avium*) were mapped. High *CPR*s occur in counties of the north-midlands and north-west, and in Northumberland; there are also scattered areas in the south with high *CPR*s. Counties of the English Plain and north-east have lower *CPR*s; these areas have relatively lower rainfalls. Factors considered to affect *CPR* distribution include differences in incompatibility, flower characters, bird damage, frost hardiness, and resistance to bacterial canker. Geology of underlying rocks is not so important as with apples.

Wild diploid apple occurs throughout Britain and Ireland, except for more northerly counties of Scotland. Wild self-incompatible diploid *P. avium* occurs throughout Britain except in northernmost Scottish counties and along the north and east of Ireland. Wild self-compatible tetraploid *P. cerasus* is restricted to southern England, Wales and some counties of the north-west, although it grows throughout the wetter districts of Ireland, except for two counties.

The extent to which self-fertility of polyploids affects distribution was evaluated. Although relatively more polyploid fruit-trees are grown in northern England, there is no county correlation between *CPR*s of apples and cherries. This indicates that fertility factors are swamped by other influences directly or not resulting from polyploidy.

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INTER-POPULATION HYBRIDS IN *DROSOPHILA MELANOGASTER*[1]

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Until recently the gene pool of a population was regarded as a relatively uniform assemblage of "wild-type" genes with a scattering of recessive mutant alleles at various loci; a precise description of a gene pool of this type and of the population of individuals carrying it at any instant could be formulated in terms of gene frequencies (the *allelotype* of Strandskov, 1950). The concept of coadaptation (Dobzhansky, 1950) pointed up the role of selection as an integrating force consolidating intra-population genetic systems on the basis of specific interactions between their different parts. In its original form coadaptation referred to chromosomal inversions within local populations of *D. pseudoobscura*. Evidence that selection operates within populations to establish heterozygosis through the retention of multiple allelic series at many loci (Wallace *et al.*, 1953; Dobzhansky and Wallace, 1953) has led to the more inclusive concept that coadaptation involves the entire gene pool. Coadaptation can be defined, then, as the mutual adjustment of interacting alleles within the gene pool of a Mendelian population brought about (a) by selection of heterozygous genotypes possessing superior adaptive values, and (b) by the occurrence of genetic recombination between successive generations. An excellent treatment of the theoretical aspects of this problem has been given by Lerner (1954) in a discussion of genetic homeostasis.

Experimental evidence for the existence of specific gene interactions responsible for phenotypic traits of individuals of isolated or local populations has been reported in the viability studies of Wallace *et al.* (1953), Vetukhiv (1953, 1954), and Brncic (1954), and studies on DDT resistance by King (1955). The demonstration rests in each case upon the contrasts between interactions observed in intra- as opposed to inter-population gene combinations.

The experiments reported in this paper deal with the ability of *D. melanogaster* larvae to develop under near-starvation conditions. The original purposes for the study were two. Both Vetukhiv and Brncic reported that inter-population hybrids of *D. pseudoobscura* were heterotic. Would a cosmopolitan species such as *D. melanogaster* with a worldwide exchange of populations owing to accidental transport by man also exhibit inter-population heterosis? If heterosis is exhibited by inter-population hybrids, what is its genetic basis? Two experiments were made. The results of the first posed an additional problem for the second: Are the gene interactions observed in inter-population crosses ascribable to the disruption through crossing-over of an intrachromosomal organization established originally by natural selection?

MATERIAL AND METHODS

The basic techniques of the two experiments were the same. Wild type *D. melanogaster* of diverse geographic origins were mated systematically for several generations to obtain larvae carrying a variety of gene combinations. Definite numbers of such newly hatched larvae were transferred to vials containing measured amounts of food and the numbers of adults hatching in the vials were recorded.

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Different populations of flies were used in the two experiments. In the first, flies were obtained from our experimental populations number 1 and number 3 (described in Wallace and King, 1951), from an Amherst, Mass. population (M) and from a Syosset, N. Y. population (S). Populations number 1 and number 3 were originally derived from an Oregon-R strain but had been maintained separately for nearly 115 generations. Population M had been kept in an experimental cage for two or three generations at the time we obtained our sample; population S had been in the laboratory for about 35 generations.

In experiment II, flies from five populations were used. Four of these were natural populations from the following places: (1) a fruit store in Blacksburg, Virginia; (2) a citrus grove at Riverside, California; (3) a vineyard near Santiago, Chile; and (4) a fruit orchard near Jerusalem, Israel. The fifth population was the Syosset, N. Y. strain maintained in a laboratory cage at this time for 45 generations.

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The matings used were similar in the two experiments. The purpose was to obtain larvae carrying definite proportions of genes from different populations in a variety of combinations. A brief account describing experiment II will serve to describe the first as well. The original flies consisted of 12 strains (*a-l*) from each of the five populations (1-Virginia, 2-California, 3-Chile, 4-Israel, and 5-New York). Each strain was started with 1–3 fertilized females captured in the "field." The following crosses were made between strains designated by the same letters: 1×2, 1×3, 1×4, 1×5, 2×3, 2×4, . . . 3×5, 4×5, 1×1, 2×2, 3×3, 4×4, and 5×5 (15 crosses, 12 bottles (*a-l*) of each). The next step entailed setting up an F_2 from each of the 10 types of inter-population crosses and, using the intra-population cultures, in remaking the same series of 15 crosses as in the first step. The F_2 series served to introduce cross-over chromosomes from hybrid females into males. The third cross consisted of mating F_2 males with hybrid females of the corresponding type [$(1a \times 2a)$ F_2 ♂♂ × $(1a \times 2a)$ ♀♀, etc.] and using the intra-population cultures (1×1, 2×2, etc.) for setting up once more the same series of 15 crosses as described in the first step. The intra-population cultures of this generation, however, were made *between* strains ($1a \times 1b$, $1b \times 1c$, $1c \times 1d$ . . . $1f \times 1a$, $1g \times 1h$, $1h \times 1i$, . . . $1l \times 1g$). The cross of F_2 males with hybrid females introduced another series of crossover chromosomes into the males; the F_3 males hatching from these cultures carried approximately 75% chromosomes from hybrid females and 25% chromosomes passed down through males with no recombination. These males were considered equivalent to hybrid females and were used interchangeably with these females whenever recombinant chromosomes were desired. The series of 12 bottles for each type of cross were now divided into two groups; strains *a* through *f* were designated *A* and strains *g* through *l*, *B*. For each cross in the next step males were taken from one of these groups and females from the other; this precaution insured the maximum heterozygosity in crosses where both parents carried genes from the same population.

The flies ultimately available for mating were F_3 hybrid males that carried crossover chromosomes, F_1 hybrid females that would transmit crossover chromosomes in their gametes, F_1 hybrid males carrying mixtures of chromosomes from two different populations but which could transmit only non-crossover chromosomes, and intra-population males and females carrying random combinations of chromosomes from their respective localities.

The final matings differed between the

two experiments. In the first where only four populations (1, 3, M, and S) were involved, all 55 possible matings were made between hybrid and non-hybrid flies: 11 × 11, [= (1 × 1) × (1 × 1)], 11 × 13, 11 × 1M, 11 × 1S, 11 × 33, 11 × 3M, 11 × 3S, . . . MS × MS, MS × SS, and SS × SS. In this series F_3 males and F_1 females were considered equivalent; the 55 matings were tabulated as indicated and the type used as males alternated, with minor exceptions, from left to right in successive crosses. Six additional matings were made using F_1 males: 13 × 13, 1M × 1M, 1S × 1S, 3M × 3M, 3S × 3S, and MS × MS.

In the second experiment more emphasis was placed on the use of F_1 males. In this experiment it was impractical to study all possible combinations. Those studied in the series utilizing F_3 males (or hybrid females) were of the following types: 11 × 12 and all other crosses of a non-hybrid of one population with a hybrid between that population and another, 11 × 23 and all other crosses between a non-hybrid of one population and a hybrid between two other populations, and 12 × 34 and all comparable "double-cross" hybrids. Besides these crosses there were those in which hybrid parents were not used: 11 × 11 and other intra-population crosses and 11 × 22 and similar inter-population crosses. There were 80 different kinds of crosses in this series. The second series made use of F_1 hybrid males. The crosses studied were 12 × 12 and all other crosses of F_1 males and females, 12 × 33 and all corresponding crosses of hybrid males with non-hybrid females of an entirely different population, and 12 × 34 and all comparable crosses of hybrid males with hybrid females of two other populations. There were 70 kinds of crosses in this series. In both experiments the final crosses were designated by code numbers to conceal their nature until collection of the data was completed.

The next aspect of the experimental technique involves larval collection and the transfer of larvae to vials. In the first experiment with only 61 kinds of crosses, 4 replicate sets of 61 mating vials were set up with 7–15 females and males in each. Wooden spoons with regular yeasted Drosophila medium were inserted each morning and the flies were allowed to oviposit for 24 hours. Four workers transferred larvae from the spoons to vials with needles. Each worker dealt with one set of 61 crosses each day, exchanging the old spoon for a fresh one in every vial and then transferring 40 freshly hatched larvae from each of the 61 spoons into an appropriately labelled vial. Each person took a different set of vials every day until the fifth and last day when he worked once more with his original set. At the end of the transferring period there were, consequently, 20 vials (4 persons × 5 days) of 40 larvae each for each of the 61 crosses.

Collecting and transferring larvae for all 150 vials of the second experiment was more than one person could do in a day. These vials were divided into two replicate groups of 80 vials each (F_3 males) and another two of 70 vials each (F_1 males). Ten–twenty males and females were placed in each mating vial. The procedure for gathering larvae was the same as in the first experiment. Transfers were made, however, by six persons for 4 days. Only 25 larvae were transferred into each vial. With six persons transferring from four groups of vials (two of 80 and two of 70) it was necessary for two pairs of persons to work on two of the groups each day. At the end of the four days, each of the six persons had transferred larvae from every one of the 150 vials of each of the two replicate sets. There were, then, 12 vials of 25 larvae each for each of the 150 crosses.

The type and quantity of medium to which the larvae were transferred also differed in the two experiments. In the first, 1″ × 4″ shell vials containing 2 cc. of cornmeal-molasses-agar medium with Tegosept-M (a mold preventative) were used. Live yeast was transferred with the larvae and, despite the fact that the food dried considerably, this amount of food

was more than ample to support most of the developing larvae. In the second experiment the medium consisted of 1 part blackstrap molasses : 7 parts of 1½% agar-water solution. Propionic acid (0.5%) was added to inhibit the growth of the live yeast which clung to the transferred larvae. The vials were ½″ × 1¾″ shell vials; each vial contained 2 cc. of the molasses-agar medium.

In both experiments the vials with developing larvae were kept at 25°. In the first experiment, counts were made on the 11th, 15th, and 17th days. The total hatch obtained by the 17th day was so large that the results are virtually meaningless; the discussion of the data will deal exclusively with the number of adults which hatched on and before the 11th day. The figures obtained reflect primarily the relative developmental rates of the different types of larvae. In the second experiment development was very slow; counts were made on the 22nd, 26th, and 28th days. For some unknown reason—possibly the propionic acid was omitted from the medium or traces of brewers yeast contaminated the agar—the development in one set of vials was noticeably better than in the others. These vials were counted only on the 20th and 24th days; nevertheless, 20 of the 1800 vials of this experiment went into second generation. The 20 vials giving more than 25 adults are scattered throughout 6 of the 8 large groups we wish to compare and so these counts are left in the data. Finally, in the first experiment, data from 3 of the 1220 vials are missing—no larvae in one, flies escaped from the second, and an obvious contamination of the third; data for these vials were completed by a technique for supplying missing information described in Snedecor (1946, p. 274).

It will be useful at this point to discuss definitions, methods of classification of larvae, and the analysis of the data. It is obvious from the nature of the crosses described that the tested larvae can be described in several ways: (1) whether loci on homologous chromosomes carry alleles from the same or different populations, (2) whether genes at loci along a single chromosome have come from the same or different populations, (3) whether different chromosomes, although not cross-over chromosomes themselves, have come from the same or different populations.

We will refer to individuals carrying alleles from different populations at homologous loci as "heterozygous"; this is strictly an operational use of this term and quotation marks will be used to emphasize this fact. Although it is impossible to specify which loci are "heterozygous" in all cases, it is possible to speak of the average proportion of "heterozygosity" for any type of cross: 11 × 11, 0% "heterozygous"; 11 × 12, 50% "heterozygous"; 12 × 34, 100% "heterozygous"; 12 × 33, 100% "heterozygous"; 12 × 13, 75% "heterozygous"; etc. It must be re-emphasized that there was no inbreeding in this material; in every instance in which two alleles from the same population occupied homologous loci, these alleles came from different strains of that population. Chromosomes transmitted by hybrid *inter-population* females or F_2 males will be referred to as "derived" chromosomes. This term emphasizes the fact that these chromosomes are recombinants between chromosomes of different populations—a recombination that could not have occurred in natural populations. A chromosome that is not "derived" is, consequently, "natural." For simplicity we will assume that all chromosomes transmitted by hybrid females or F_2 males are crossover chromosomes; the tested larvae can be classified according to the proportion of derived chromosomes they carry: 11 × 11, 0% derived; 11 × 12, 50% derived; 11 × 22, 0% derived; 12 × 34, 100% derived, etc.

Through common usage the term "heterozygous" is frequently used in reference to individuals rather than to the genes carried by these individuals; we will use the term "derived" in the same way and will occasionally refer to "derived" individuals.

Finally, in those crosses involving F_1 hybrid males, larvae are formed that have received from their fathers mixtures of natural chromosomes from two different populations—derived haploid sets: 12 F_1 ♂ × 33 ♀♀, 100% "heterozygous," 0% derived chromosomes, derived haploid set from father; 12 F_1 ♂ × 34 ♀♀, 100% "heterozygous," 50% derived chromosomes (from hybrid mother), derived haploid set from father; 12 F_1 ♂ × 12 ♀♀, 50% "heterozygous," 50% derived chromosomes, derived haploid set from father; etc.

The primary importance of the data for our present purpose lies in contrasting the effect of "heterozygosity" and of recombination (measured by derived chromosomes and derived haploid sets) on the survival of larvae under starvation conditions. In our statistical treatment of the data we have not attempted a complete analysis of variance; instead, we have obtained estimates of experimental error and have used these estimates in comparing the average survival of larvae classified according to proportions of "heterozygous" loci and derived chromosomes. The estimate of error in the first experiment was based on the variance between replicated sets; the estimate in the second experiment was based on the variance within all 12 vials of the two replicated sets. In each case the estimated error is too large but these estimates are accurate enough for our purposes.

A schematic representation of the chromosomal constitution of the different classes of flies synthesized in the two experiments is shown in figure 1. In each instance only one of many possible combinations of populations is shown (for instance, six different crosses in experiment I and ten in experiment II gave rise to

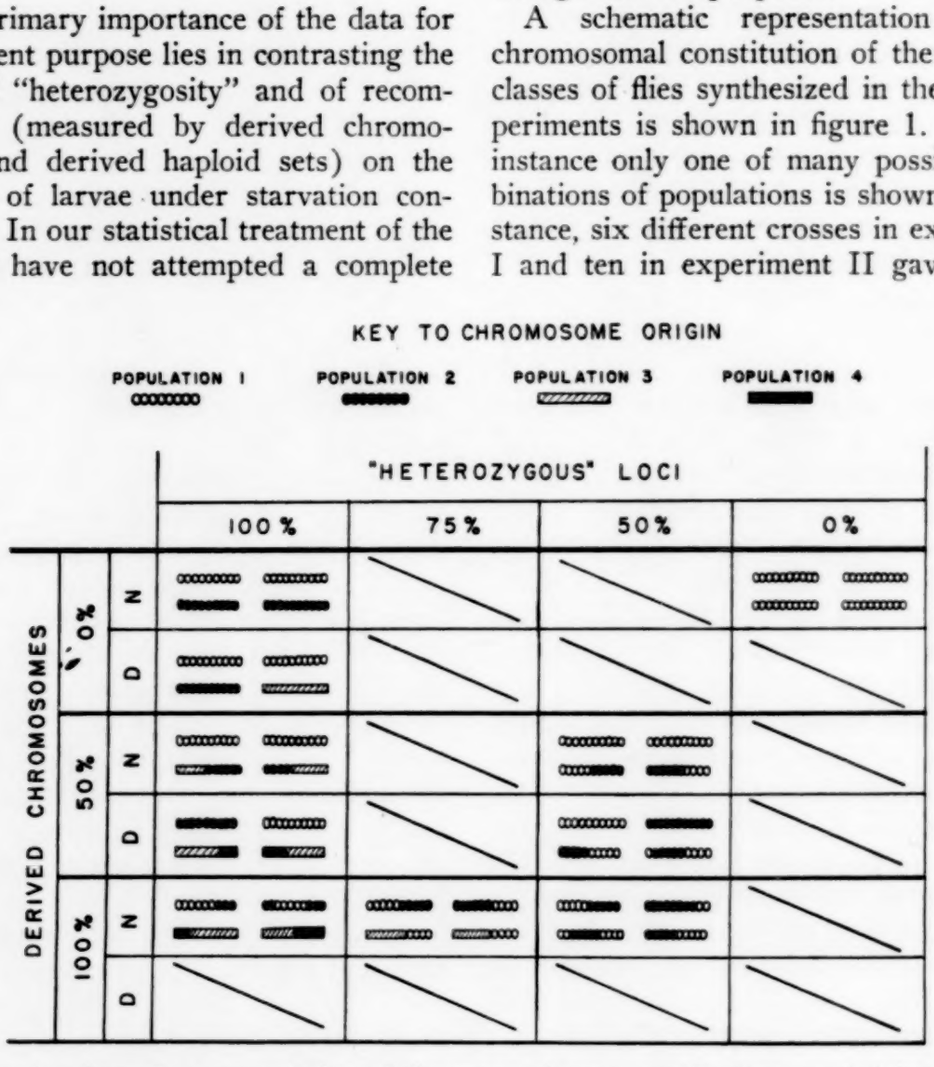

FIG. 1. A schematic representation of the types of gene combinations carried by the flies developing under starvation conditions. Each diagram represents the diploid number of two chromosomes. "Heterozygous" loci gives for each class of flies the average proportion of loci carrying alleles from two different populations. "Derived chromosomes" gives the approximate proportion of chromosomes which carry genes from two different populations (crossover chromosomes from inter-population hybrid females or F_2 males). Derived and natural haploid sets are indicated D and N; a derived haploid set is a combination of non-crossover chromosomes from two different populations obtained from an F_1 hybrid male.

100% "heterozygous," 0% derived individuals with natural haploid sets). It has been possible to indicate only one combination of genes in those classes possessing derived chromosomes or derived haploid sets; it must be remembered that an almost infinite variety of combinations is possible in these instances.

Results and Conclusions

Maternal Effect

In evaluating the effects of different genotypes on the survival of larvae under adverse conditions, it is necessary to eliminate any advantage gained by larvae through the genotypes of their mothers. In a preliminary analysis (Wallace, 1954b) of the data from experiment I, the existence of a maternal effect was not detected. A more careful analysis of data from experiment II revealed such an effect and re-examination of the earlier data revealed it there as well.

The detection of a maternal effect depended upon the fact that in certain types of crosses only one hybrid parent was involved; this parent was either an F_1 hybrid female or an F_2 male. The data were grouped so that one population was considered at a time and the average number of adults hatching in crosses involving "homozygous" females was compared with the number hatching in analogous crosses using "heterozygous" females. The results of these tests were as follows: Experiment I: $\bar{d}$ (the average difference) $= 5.145$, $\sigma_{\bar{d}} = 2.25$, $\bar{d}/\sigma_{\bar{d}} = 2.29$, degrees of freedom $= 55$, p = probability $= .02 - .03$. Experiment II: $\bar{d} = 10.28$, $\sigma_{\bar{d}} = 4.78$, $t = 2.15$, d.f. $= 12$, $p = .05$. In both instances the chances that the observed differences are due to chance are small; the agreement between the experiments makes it almost certain that a hybrid maternal genotype increases the chances of survival of developing larvae. Further analysis failed to reveal any indication that the maternal effect was limited only to larvae of certain genotypes or that only some hybrid females produced the effect. Consequently, correction factors for crosses involving homozygous female parents were calculated from the analyzed data; these were 1.04 for experiment I and 1.06 for experiment II. Squares of these factors were used in calculations involving variances.

Following the adjustment for maternal effects, the data of experiment II were examined once more for evidence of interactions between the X and Y chromosomes and the cytoplasm when these were of different origins. No evidence for such interactions was found.

Larval Viability

The total numbers of survivors in all tested combinations are given in tables 1 and 2. Data on the number of survivors in individual vials are too voluminous for publication. Figures in the tables which have been corrected for maternal effect (nearest whole fly) are marked by an asterisk. The data for experiment I represent counts made on the 11th day after the transfer of larvae.

In tables 3 and 4 the crosses listed in tables 1 and 2 have been grouped on the basis of "heterozygosity," derived chromosomes, and derived haploid sets. The mean number of surviving adults and the error of the mean are shown for each class. Pairs of classes which differ significantly ($p < .05$) are indicated by arrows in figures 2 and 3; the arrows point toward the class with the fewer survivals.

In general the experiments agree reasonably well. The striking aspect of both figures 2 and 3 is the downward sweep of the arrows. This pattern is especially noticeable in figure 2 in which the arrows fan down from the two upper corners. A combined summary of the direction of the arrows in the two experiments is given in table 5. Twenty-seven of 32 arrows point downward; the greater the proportion of recombined chromosomes, the smaller the numbers of surviving individuals. "Heterozygosity" is not nearly as

TABLE 1. *The 61 different combinations of genes from populations 1, 3, M, and S (see text for origin of these flies) studied in experiment I*

The combinations are grouped according to proportion of "heterozygous" loci (H) and proportion of derived chromosomes (d) as explained in text. The group indicated by $F_1 ♂$ possesses a derived haploid set of non-recombinant chromosomes. n = the number of flies hatched by the 11th day, a measure of relative developmental rates. The original number of larvae in each instance was 800 (20 vials, 40 larvae each).

	n
0% H, 0% d	
11×11	631*
33×33	511*
MM×MM	588*
SS×SS	640*
100% H, 0% d	
11×33	615*
11×MM	643*
11×SS	636*
33×MM	602*
22×SS	599*
MM×SS	610*
100% H, 50% d	
11×3M	571*
11×3S	544
11×MS	591
13×MM	558*
13×SS	508*
1M×33	517
1M×SS	593*
1S×33	574*
1S×MM	567
33×MS	674*
3M×SS	544*
3S×MM	482*

	n
100% H, 100% d	
13×MS	561
1M×3S	593
1S×3M	533
75% H, 100% d	
13×1M	566
13×1S	621
13×3M	559
13×3S	587
1M×1S	528
1M×3M	521
1M×MS	494
1S×3S	522
1S×MS	524
3M×3S	546
3M×MS	521
3S×MS	528
50% H, 100% d	
13×13	499
1M×1M	543
1S×1S	550
3M×3M	553
3S×3S	516
MS×MS	541

	n
50% H, 50% d	
11×13	580*
11×1M	604
11×1S	586*
13×33	566
1M×MM	567
1S×SS	607
33×3M	514*
33×3S	470
3M×MM	556*
3S×SS	603*
MM×MS	603
MS×SS	577
50% H, 50% d ($F_1 ♂$)	
13×13	524
1M×1M	568
1S×1S	610
3M×3M	579
3S×3S	537
MS×MS	596

* Adjusted for maternal effect (×1.04).

important; of the 32 arrows indicating significant differences in survival, 10 point to the left and 10 to the right. Whatever the differences between the genetic systems of the various tested populations, these differences are manifested much more by recombination than by inter-population "heterozygosity."

Finally, inter-population hybrids (100% "heterozygous," 0% derived, natural haploid sets) on the average do exhibit heterosis in this species. This is certainly true with the five populations studied in experiment II and is quite probably true for the four of experiment I as well although the difference here was not statistically significant ($p = .08$).

Thus, answers to the questions listed earlier are already available. In spite of the accidental transport of large numbers of individual *D. melanogaster* from one part of the globe to another, local populations have diverged genetically. This is indicated by the difference between intra- and inter-population crosses. It is re-emphasized by the average decrease in viability that accompanies inter-population recombination. Not only have the original populations diverged but this divergence has involved the formation within these populations of mutually compatible gene combinations.

The data presented above lead to certain conclusions regarding the genetic basis of inter-population heterosis. Vetukhiv (op. cit.) suggested that heterozygosity

TABLE 2. *The 150 different combinations of genes from five natural populations (1, Virginia; 2, California; 3, Chile; 4, Israel; 5, New York) studied in experiment II grouped according to proportion of "heterozygous" loci (H) and derived chromosomes (d)*

Those groups indicated by $F_1♂$ possess a derived haploid set of non-recombinant chromosomes. n = the total number of flies hatched. The original number of larvae in each instance was 300 (12 vials, 25 larvae each).

	n
0% H, 0% d	
11×11	173*
22×22	154*
33×33	166*
44×44	176*
55×55	111*
100% H, 0% d	
11×22	218*
11×33	145*
11×44	222*
11×55	121*
22×33	176*
22×44	216*
22×55	174*
33×44	173*
33×55	134*
44×55	139*

	n
100% H, 50% d	
11×23	161
11×24	153*
11×25	162
11×34	153
11×35	170*
11×45	146*
12×33	184*
12×44	217
12×55	176
13×22	174*
13×44	146*
13×55	138*
14×22	212
14×33	230*
14×55	140
15×22	172*
15×33	164
15×44	161
22×34	172
22×35	173*
22×45	146*
23×44	192*
23×55	139*
24×33	133
24×55	158*
25×33	159
25×44	184
33×45	172*
34×55	162*
35×44	110

	n
100% H, 100% d	
12×34	171
12×35	105
12×45	154
13×24	131
13×25	198
13×45	131
14×23	152
14×25	158
14×35	151
15×23	135
15×24	134
15×34	173
23×45	140
24×35	130
25×34	138
50% H, 50% d	
11×12	159*
11×13	171
11×14	152*
11×15	146
12×22	153
13×33	203
14×44	169*
15×55	128*
22×23	141
22×24	193*
22×25	193
23×33	170
24×44	156
25×55	163*
33×34	167
33×35	218
34×44	138
35×55	112*
44×45	145
45×55	143*

	n
100% H, 0% d ($F_1♂$)	
12×33	242*
12×44	164*
12×55	106*
13×22	155*
13×44	146*
13×55	158*
14×22	145*
14×33	126*
14×55	130*
15×22	130*
15×33	173*
15×44	131*
23×11	173*
23×44	163*
23×55	136*
24×11	173*
24×33	170*
24×55	174*
25×11	137*
25×33	110*
25×44	179*
34×11	202*
34×22	190*
34×55	119*
35×11	164*
35×22	167*
35×44	172*
45×11	200*
45×22	144*
45×33	195*

	n
100% H, 50% d ($F_1♂$)	
12×34	159
12×35	116
12×45	123
13×24	176
13×25	129
13×45	109
14×23	159
14×25	194
14×35	128
15×23	149
15×24	139
15×34	147
23×14	172
23×15	180
23×45	160
24×13	161
24×15	134
24×35	117
25×13	133
25×14	155
25×34	146
34×12	175
34×15	160
34×25	135
35×12	203
35×14	90
35×24	159
45×12	139
45×13	167
45×23	139
50% H, 50% d ($F_1♂$)	
12×12	217
13×13	154
14×14	128
15×15	139
23×23	175
24×24	140
25×25	172
34×34	177
35×35	150
45×45	138

* Adjusted for maternal effect (×1.06).

per se was responsible for the heterosis of F_1 hybrids observed in his experiments since selection can not operate to coadapt the genetic systems of two isolated populations. However, it is quite apparent from figures 2 and 3 that heterozygosity alone does not explain the heterosis found in our material. Eight classes of individuals tested in the two experiments are 100% "heterozygous"; of these only the inter-population hybrids (100% "heterozygous," 0% derived) are consistently

TABLE 3. *Mean number of flies present at 11th day in vials of experiment I grouped on the basis of proportion of "heterozygous" loci and derived chromosomes*

These figures are only 1/4th as large as those in table 1 because the analysis of variance was based on four replicated sub-experiments. For comparative purposes the material is presented in the same form as figure 1 even though certain classes of individuals were not tested in this experiment.

			"Heterozygous" loci			
			100%	75%	50%	0%
derived chromosomes	0%	N	154.38 ±2.34			148.13 ±2.91
		D	not tested			
	50%	N	140.06 ±2.20		142.36 ±2.17	
		D	not tested		142.25 ±3.10	
	100%	N	140.58 ±3.96	135.77 ±2.00	133.42 ±3.53	
		D				

more viable than flies from local populations. The difference between the F_1 hybrids and the others lies in the amount of recombination that has occurred. The F_1 hybrid carries two haploid sets obtained intact from local populations; the others possess at least one haploid set in which genes from two populations have been recombined. Heterosis in our material must, then, be ascribed to heterozygosity for selected gene complexes rather than mere heterozygosity. The large drop in the number of survivors among individuals 100% "heterozygous," 100% derived relative to those 100% "heterozygous," 50% derived (table 4) indicates that one "natural" haploid set is capable of functioning quite well in the presence of one which carries genes from two different populations This effect is not evident, however, in the data from experiment I (table 3)

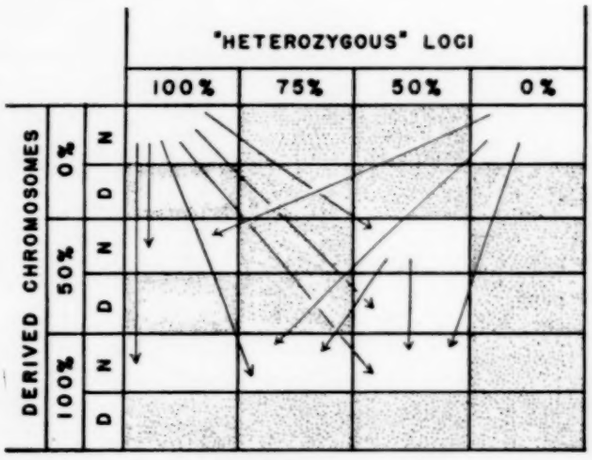

FIG. 2. A diagrammatic representation of the significance of the figures given in table 3. Each arrow is drawn between classes that differ significantly ($p < .05$) in the number of flies hatched by the 11th day. The arrow points from the class with the greater number to the one with the lower number. Stippled areas denote classes not subject to study by the experimental technique used or (partially stippled) classes not included in experiment I.

TABLE 4. *Mean number of flies in final counts of vials in experiment II grouped on the basis of proportion of "heterozygous" loci and derived chromosomes*

These figures are equivalent to those of table 2 since the analysis and estimate of error was based on the 12 vials of each combination. For comparative purposes the material is presented in the same form as figure 1 even though certain classes of individuals were not tested in this experiment.

			"Heterozygous" loci			
			100%	75%	50%	0%
derived chromosomes	0%	N	171.80 ±1.94			156.00 ±2.95
		D	159.13 ±1.13			
	50%	N	165.30 ±1.17		161.00 ±1.52	
		D	148.43 ±1.05		159.00 ±1.90	
	100%	N	146.73 ±1.61	not tested	not tested	
		D				

Among the types of flies tested in experiment I were six crosses in which F_1 hybrid males were used as parents. Flies obtained by these crosses were 50% "heterozygous" and 50% derived just as those of one other class in this experiment. The two classes differed in the presence of a natural haploid set in the

individuals of one class and a derived haploid set in the other. The mean numbers of flies counted in the two classes were nearly identical, a fact which led to the suspicion that inter-chromosomal interactions were relatively unimportant in determining developmental rates. To test this possibility a greater emphasis was placed on the use of F_1 males in the second experiment. The answer is immediately apparent: Inter-population hybrids carrying two different haploid sets (100% "heterozygous," 0% derived) are more viable than individuals carrying a haploid

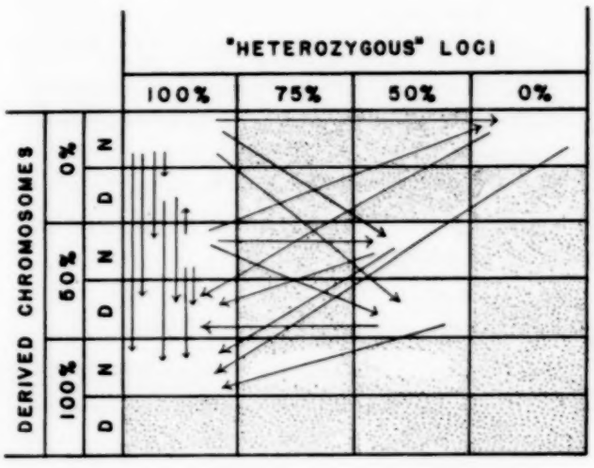

FIG. 3. A diagrammatic representation of the significance of the number of survivors given in table 4. As in figure 2 each arrow connects two classes that differ significantly ($p < .05$) and points toward the class with the smaller number. Stippled areas denote classes impossible to form by the technique used or (partially stippled) classes not included in experiment II.

set from one population and a second set consisting of chromosomes from two different populations even though these chromosomes have segregated without crossing over. Inter-chromosomal interactions, therefore, do influence the viability of hybrids. The existence of this type of interaction renders the experimental technique useless for demonstrating the "organization effects" suggested by experiment I. This does not imply that such effects do not exist (see Levitan, 1954) but that their demonstration requires another type of experimental analysis.

TABLE 5. *An analysis of the directions in which the arrows of figures 2 and 3 point*

"Heterozygosity" decreases from left to right; the amount of recombination increases from top to bottom.

	Right	Vertical	Left	Total
Up	1	1	0	2
Horizontal	2	xxxxxx	1	3
Down	7	11	9	27
Total	10	12	10	32

In some respects the results of the two experiments differ. In experiment I, for instance, the two classes of flies, 100% heterozygous–50% derived and 100% heterozygous–100% derived, did not differ significantly while the same classes in experiment II differed markedly. Furthermore, in experiment I more flies were counted among intra-population crosses than among those 100% heterozygous, 50% derived, natural haploid set; just the reverse was found in experiment II. In part these differences must reflect the

TABLE 6. *The probability that the observed differences in survival of flies of various genotypes would have occurred were the gene pools of different populations actually identical*

These results are based on the survival of larvae in a series of crosses in which genes from two populations have been combined in comparable ways with genes from other populations.

Experiment I

	1	3	M	S
1	x	.03	.07	.90
3	.03	x	.01	.015
M	.07	.01	x	.75
S	.90	.015	.75	x

Experiment II

	1	2	3	4	5
1	x	.13	.57	.80	.05
2	.13	x	.03	.09	.003
3	.57	.03	x	.18	.90
4	.80	.09	.18	x	.01
5	.05	.003	.90	.01	x

use of different populations, different media, and the analysis of two different characters (developmental rate and total survival). The differences between the two experiments probably reflect, too, some shortcomings of the experimental technique. Some classes of larvae were genetically uniform while those in other classes were genetically variable. Thus, where recombination had occurred, a variety of genotypes was produced; the F_1 hybrids were, of course, more or less uniform. The effect of population density on survival among homogeneous and heterogeneous collections of larvae could easily differ. For these reasons, the rather high survival in experiment II of individuals 100% "heterozygous," 50% derived, carrying a natural haploid set of chromosomes is probably misleading. As mentioned above, the relation between this class and the 0% "heterozygous," 0% derived class is reversed in the two experiments. Furthermore, this same class (100% "heterozygous," 50% derived) has more survivors than the class 100% "heterozygous," 0% derived, with a derived haploid set; if real, this difference is difficult to explain. In view of these discrepancies, it seems doubtful that the apparent decrease in viability accompany a decrease in heterozygosity between the class 100% "heterozygous," 50% derived, natural haploid set, and the class 50% "heterozygous," 50% derived, natural haploid set reflects the real situation.

Individuals 50% "heterozygous," 50% derived, with derived haploid sets were more viable than individuals 100% "heterozygous," 50% derived, with a derived haploid set. The *decrease* in viability accompanying this *increase* in heterozygosity is probably real. First, the difference is large. Second, the number of survivors in each of these classes compares well with that found in a corresponding class involving the same combination of populations. In this instance, the 50% "heterozygous" individuals are obtained by inbreeding F_1 males and females. Only two populations are involved in any one of the ten kinds of crosses included in this class. Through the union of gametes carrying complementary portions of important gene combinations, it is possible that a number of larvae obtained genotypes substantially similar to those of either F_1 hybrids or intra-population flies. This could easily be the case in our experiments since naturally occurring inversions were not eliminated from the material used. (Contrast our inter-population F_2 flies with those of Vetukhiv who worked with inversion-free material; in his case the inter-population F_2 individuals were less viable than the intra-population flies.) There is virtually no opportunity for larvae of the 100% "heterozygous" class to possess genotypes essentially similar to those of either F_1 hybrids or intra-population individuals. This is so because the 100% "heterozygous" class involves portions of the gene pools of *four* rather than *two* different populations.

Comparing Gene Pools

A type of analysis for which these data are useful is the comparison of gene pools of different populations. In the past such comparisons have been based primarily on frequencies of recessive lethal or visible mutations. This type of study has of necessity dealt with chromosomes in the homozygous condition. There are several interpretations possible when significant differences are found. Assume, for instance, that one population of *D. melanogaster* is found to have 20% second chromosome lethals and another to have 30% and that this difference is significant. This difference will suggest to one person a difference in effective population size and a greater accumulation of lethals in the larger one. Another person might consider mutation rates as the causative factor. A third will make assumptions regarding the number of lethal loci, frequencies of lethals at each locus, allelism of these lethals and will conclude—all other things being equal—that the fitness of one population is slightly less that of

the other. A fourth might regard this difference as merely an overt manifestation of fundamental differences in genetic modifiers, polygenic complexes—the whole genetic network—upon which the populations are built. Except in the last instance, a tacit assumption is that the nonlethal alleles at various loci are alike in the two populations and that the information on lethals offers a substantial basis for inter-population comparisons. The experiments discussed in this paper offer a much better opportunity for comparing gene pools of different populations—comparisons based on the interactions between substantial segments of these pools when in novel combinations. For instance, to compare populations 1 (Virginia) and 2 (California) of experiment II we can compare the viability of the following classes of larvae: 11 × 11 vs. 22 × 22, 11 × 33 vs. 22 × 33, 11 × 34 vs. 22 × 34, 14 × 35 vs. 24 × 35, etc. There are seventeen comparisons one can make between any two populations of experiment I; forty in experiment II. The assumption in each instance is that the two populations are alike; using the difference in the number of survivors and the error of this difference, the probability of obtaining this difference by chance can be computed. These individual probabilities can be combined by Fisher's method ($-2\Sigma \ln p_i = \chi^2$ with 2N degrees of freedom, where N = number of comparisons and p_i = any of the individual probabilities); the result is the probability of observing the entire array of probabilities through chance alone. The finding of a maternal effect changes the results substantially from those published earlier (Wallace, 1954b). The summarized results of these experiments are given in table 6. It is apparent that in about one half of all comparisons in either experiment, the two populations are found to differ significantly. There are indications, too, that these differences reflect specific interactions between the genes of different populations: In experiment I, 1 = S ($p = .90$) and M = S ($p = .75$) but the probability that 1 = M is only .07; in experiment II, 1 = 2 ($p = .13$) and 1 = 3 ($p = .80$) but 2 does not equal 3 ($p = .03$); 3 = 4 ($p = .18$) and 3 = 5 ($p = .90$) but 4 does not equal 5 ($p < .01$). These data offer additional support to the hypothesis that alleles are selected within populations on the basis of interactions with alleles at the same and/or different loci; the phenotypic manifestations of these alleles are altered by the substitution of novel combinations for the original coadapted complexes of the local populations.

Discussion

The data presented above bear on a number of genetic and evolutionary problems. The discussion of these data will be limited to their bearing on the concept of an integrated gene pool and on heterosis.

In the first section of this paper a definition of coadaptation was given that involved two phenomena, the adaptive superiority of heterozygous individuals and the mutual adjustment of interacting alleles within Mendelian populations. The experiments have dealt solely with the second of these two phenomena; they have no direct bearing on the question of intrapopulation heterozygosity. The results clearly indicate that the gene pools of different local populations of *D. melanogaster* do consist of mutually adjusted gene combinations. These gene combinations were disrupted by inter-population gene recombination.

The presumed basis for coadaptation lies in polygenic complexes. The data indicate that the interacting complexes of the material studied include genes of different chromosomes. Data pertinent to this point were obtained through the use of F_1 inter-population hybrid males as parents in certain crosses. The chromosomes transmitted by such males to their offspring are non-crossover chromosomes but, within the haploid set transmitted, there are mixtures of chromosomes from different populations. The viability of individuals 100% "heterozygous" receiving these "derived" haploid sets was lower

than that of F_1 hybrids. Crossing-over and intra-chromosomal recombination resulted in still lower viabilities. Not only are the elements of the interacting complexes distributed between different chromosomes but those portions on individual chromosomes can be disrupted by recombination. The latter point is especially emphasized in the results of Brncic (1954) who restricted his studies to the third chromosome of *D. pseudoobscura*.

If a coadapted combination of genes occupies several chromosomes with a number of factors on each chromosome, this combination can be properly referred to as "polygenic." The concept of polygenic complexes has been questioned recently in this journal (Goldschmidt, 1953) but it looks very much as though in his denial of such complexes, Goldschmidt adequately though perhaps inadvertently described just such a gene system in the following passage: ". . . he (Dubinin) selected simultaneously and without discrimination a) for homozygosity of the simple Mendelian mutant ***net,*** b) for homozygosity of the mutant *blistered* (or *balloon* or both?) in the same chromosome, c) for the combined presence of both (or more) mutants in different homozygous or heterozygous combinations, d) for dominance enhancers, e) for multiple alleles of the loci involved (. . .), and f) for enhancers of expressivity." The necessary raw materials are all here; if we reject Goldschmidt's notion that these genes merely "float" in populations, we can assume that selection brings about a mutual adjustment of these genes and thereby produces a coadapted complex.

In a number of recent papers (Dobzhansky, 1950, 1952; Vetukhiv, 1953, 1954; Lerner, 1954) a distinction has been made between heterosis observed within Mendelian populations (euheterosis) and the hybrid vigor characteristic of inter-population or inter-specific hybridization (luxuriance). The distinction is based on the role of selection in bringing about euheterosis and the inability of selection to cause or influence luxuriance. Coadaptation can arise only within populations of interbreeding individuals.

It seems, however, that euheterosis and luxuriance can, in some instances, be referred to a common genetic basis; the data presented above help in identifying this basis. It was seen that the only class of flies that was substantially heterotic relative to the individuals of the local populations were the F_1 hybrids between these populations. These hybrid individuals carried two haploid sets of chromosomes each of which contained a combination of genes established by selection within a local, inter-breeding population. In synthesizing these hybrids no chromosomal mechanisms were provided to maintain and perpetuate these combinations and, as tables 3 and 4 show, heterosis was lost by recombination. If heterosis of integrated gene combinations were a general phenomenon, it might be expected that genetic systems capable of exploiting this type of heterosis would be established by natural selection within local populations. These systems would include chromosomal mechanisms capable of preventing recombination; the inversions commonly found in Drosophila populations are just this type of mechanism. Inversions, however, do not provide a perfect solution to the problems posed by recombination (Wallace, 1953, 1954a): Double cross-overs can remove blocks of genes from within long inversions. Certain combinations of overlapping inversions may permit a serial transfer of genes from chromosomes of one arrangement to chromosomes of a different arrangement. Inversions in one chromosome modify (increase, as a rule) the amount of crossing-over on other chromosomes. In addition to these short-comings of inversions as a mechanism for maintaining isolated blocks of genes, the populations face additional problems raised by mutation and the introduction of "alien" genes by migrant individuals from more or less remote localities. Consequently, it would seem reasonable in the case of Mendelian populations to regard euheterosis as a manage-

able fraction of luxuriance. The higher average viability of inter-population F_1 hybrids is a measure of the price paid by local populations for an integrated gene pool capable of being transmitted successfully from generation to generation.

Finally, it is interesting to contrast the conclusions based on studies of natural and artificial populations of Drosophila with those based on studies of self-fertilizing species or inbred material. Smith (1952), for instance, found that non-allelic interactions were not an important source of variation in studies on *Nicotiana*. Gowen (1952) reported a linear increase in egg production accompanying a chromosome-by-chromosome increase in heterozygosity in crosses between two inbred strains of *D. melanogaster*. The techniques used by corn breeders (that of Jenkins, for instance) to predict the yield of double-cross hybrids would be highly inefficient if recombination disrupted interacting gene combinations. In these studies, heterozygosity rather than recombination seems to play the dominant role in determining the hybrid and partial-hybrid phenotypes. The type of co-adapted gene combinations revealed by studies of Vetukhiv, Brncic, and by our own material seem to be lacking in the inbred material; either such systems were never present or they were abandoned upon the assumption of new mating patterns.

Summary

Two experiments were made to answer the following questions: (1) Do inter-population hybrids of *Drosophila melanogaster* exhibit hybrid vigor? (2) If it exists, what is the genetic basis of this heterosis? Flies from eight populations were used: two experimental, laboratory populations and six natural populations from Virginia, California, Massachusetts, New York, Chile, and Israel. The characters studied were relative developmental rates (experiment I) and survival ability (experiment II) under starvation conditions.

Through systematic matings between flies from different populations larvae were obtained that could be classified according to percentages of "heterozygous" loci and proportions of "derived" chromosomes (definitions of these terms are given in the text). Definite numbers of these larvae were grown in small amounts of food. The greatest numbers of survivors were found among the F_1 inter-population hybrids. The greater the amount of recombination (measured in terms of "derived" chromosomes or "derived" haploid sets) the smaller the number of survivors. Recombination was much more important in determining viability than was "heterozygosity."

It was concluded that heterozygosity for integrated gene complexes produces the heterosis exhibited by inter-population hybrids. Recombination destroys these complexes and, simultaneously, the heterosis dependent upon them. It was suggested that local populations of Drosophila exploit to the extent permitted by their breeding structures, heterosis resulting from heterozygosity of integrated gene combinations. Viewed in this way, euheterosis can be considered as a fraction of luxuriance that is compatible with problems concerning the transmission of these integrated systems through successive generations.

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A HYBRID WOODPECKER AND ITS SIGNIFICANCE IN SPECIATION IN THE GENUS *DENDROCOPOS*

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In the course of field work in the Sierra del Carmen, Coahuila, in April of 1953 a hybrid woodpecker was taken representing a cross between the very distinct species *Dendrocopos villosus* and *Dendrocopos scalaris*. Not only are the parent species greatly different in size, but they represent two different species groups in the North American section of the genus, the group with longitudinally striped backs and the ladder-backed group (see Voous, 1947). The only other reported hybridization between these groups is that between the small *Dendrocopos pubescens* and the somewhat larger, ladder-backed, *Dendrocopos nuttallii* (Cockrum, 1952: 146). The significance of the hybrid here reported takes these directions: (1) Some ecologic and distributional evidence is available to suggest why the biologic isolation between the parent species broke down in this instance. (2) The interplay of many pattern and size factors is open to a partial analysis. (3) The hybrid's characters suggest in many respects the combination of characters present in another species that occurs in a different geographic area.

Figures 1 and 2 and the analytic comparison to follow will serve to describe the characters of the hybrid. The bird was taken on April 12 and is a female. The ovary was considerably disrupted by shooting and although fragments of ovarian tissue could be seen, no clear evidence of breeding activity could be discerned; certainly it was not actually engaged in laying. No mate was found in the area at the time or subsequently. I was first attracted to the bird by its drumming on a tree, a form of song or advertising behavior in such woodpeckers. This performance may have indicated solitariness and search for a mate but it should be realized that female woodpeckers may do some drumming while paired and nesting. Obviously we know nothing, then, about the fertility of this female, which in the light of its combination of characters can be rather safely assumed to be an F_1 individual. Since its parents must have broken through the behavioral differences that normally separate the two species, it may be supposed that this hybrid could have done likewise, although perhaps only under especially favorable conditions of mate availability.

BREAKDOWN OF BIOLOGIC ISOLATION

The Hairy Woodpecker (*Dendrocopos villosus*) and the Ladder-backed Woodpecker (*Dendrocopos scalaris*) have limited contact although their general ranges overlap broadly. The Hairy Woodpecker occupies montane coniferous forest in the interior of the southwestern part of the continent and the Ladder-backed Woodpecker inhabits chiefly desert scrub and river valley vegetation in this region. In California, Nevada, Utah, western Arizona, Baja California, and Sonora their breeding ranges rarely are in contact as a consequence of this ecologic and zonal separation. In central Arizona, southern New Mexico, and western Texas *scalaris* ranges up into the juniper, piñon, and oak belts adjoining the montane conifers and thus is in border contact with *villosus*, as in the Guadalupe Mountains of Texas (Burleigh and Lowery, 1940: 108–109). In southeastern Arizona, Sonora, and western Chihuahua the oak belt is occupied chiefly by the very different *Dendrocopos arizonae* which ranges up also

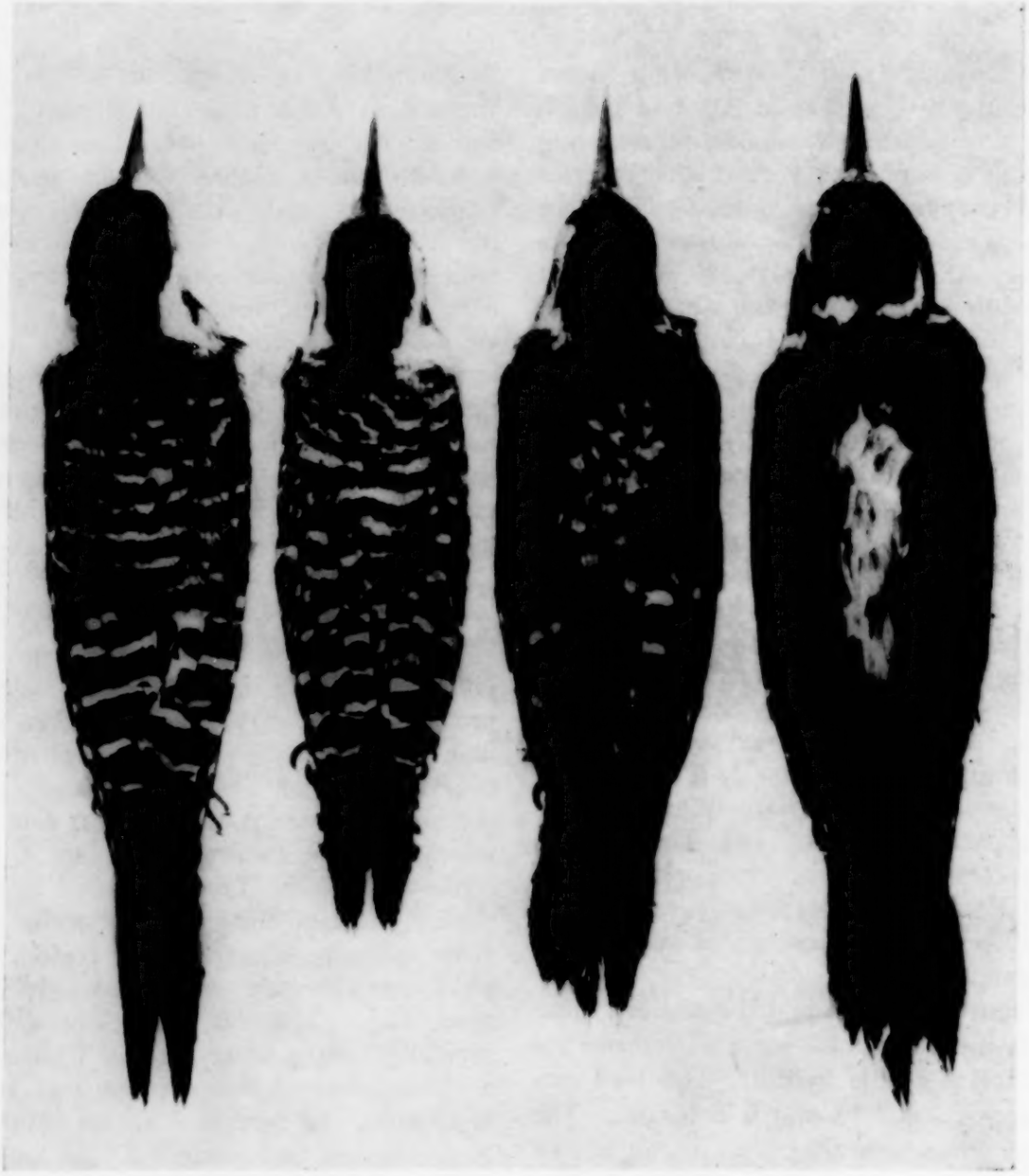

FIG. 1. Dorsal views of female woodpeckers of the genus *Dendrocopos*. Left to right: *D. borealis, D. scalaris, D. villosus* × *D. scalaris, D. villosus.*

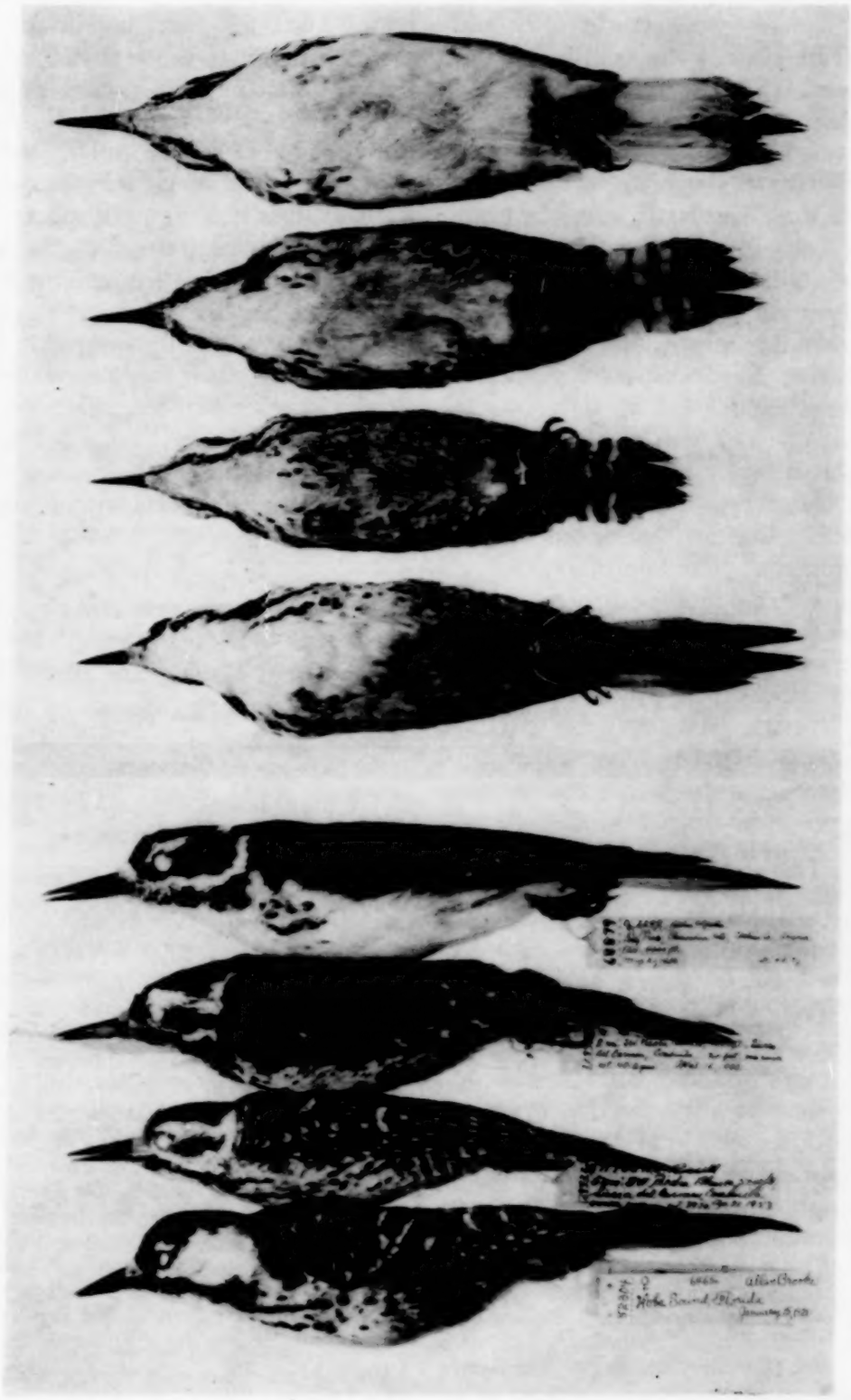

FIG. 2. Above. Ventral views of specimens shown in figure 1. Top to bottom: *D. villosus*, *D. villosus* × *D. scalaris*, *D. scalaris*, *D. borealis* right rectrix 6 of the hybrid is lacking.
Below. Side views of same specimens in same order.

into pine-oak sympatrically with *villosus*. In these areas *arizonae* may be regarded as interposed zonally between *villosus* and *scalaris*. But east of the continental divide *arizonae* is absent and it is here especially that *scalaris* ranges upward to meet *villosus*.

In the Sierra del Carmen, the high-zone conifer belt is well isolated from the nearest conifer belts of like character in other mountains. Our intensive field work of a month's duration revealed no population of *villosus* in the Sierra, although conditions seemed to be ideal for the species in the numerous valleys and mesas grown to good stands of white and ponderosa pines and Douglas fir. Presumably the species has never had a population there, or if it once existed, it has died out or has but the rarest remnants. Recolonization would have to be effected by dispersal from occupied areas 200 miles or more away to the north, west, or south, an unlikely although not impossible event in this rather sedentary species. Under these circumstances *scalaris* seems to have spread upward in the Sierra del Carmen to an unusual degree into mixed pine and oak forest at least to 6800 feet altitude.

The hybrid was taken essentially at this upper limit of *scalaris,* at 7000 feet, in a stand of live and deciduous oaks. It must have had a *villosus* parent, and we may suppose, therefore, that a vagrant or remnant of this species occurred in the higher oak and conifer habitat attractive to it. In the absence of any effective breeding population of its own kind, the barriers of behavior and species recognition, entailing different sight and sound releasers, were overcome under the drive to mate and a pairing with an available *scalaris* ensued. Under circumstances permitting normal choice of mates this probably would not occur and the two species might live sympatrically in numbers. Indeed this is what happens in western California where a ladder-backed species, *D. nuttallii,* has considerable areas of sympatry with *villosus* and where indeed the very closely related *nuttallii* and *scalaris* have a narrow zone of sympatry along the western desert border and yet have not been found crossing. What the local conditions actually were pertinent to the reported crossing of *nuttallii* and the striped-backed *pubescens* (Cockrum, *op. cit.;* Ridgway, 1886) we cannot now determine. This hybrid was stated to be from San Francisco, where *nuttallii* does not regularly occur, but this locality designation may be only general in reference to the San Francisco Bay region. Elsewhere in the vicinity *nuttallii* and *pubescens* are broadly sympatric.

In essence, then, we postulate that the insular character of the Sierra del Carmen in Coahuila, leading to faunal impoverishment, has resulted in one member of a generic group, *scalaris,* spreading out abnormally in an ecologic sense, whereupon the rare appearance in this invaded ecologic area of an essentially absent species has forced a break down of the ethologic barriers that usually are effective in separating sympatric species of the genus *Dendrocopos.*

Comparison of Characters of the Hybrid

Ventral surface. Villosus (race *icastus* of Chihuahua) is dull white except for black malar stripes, heavy black streaks at the side of the breast, and almost obsolete dusky bars on the flanks. *Scalaris* (local race *cactophilus*) is buffy white and has the same submalar stripes but the breast is extensively streaked, although less boldly, and the flanks and under tail coverts are heavily barred. The hybrid is intermediate in degree of whiteness and in the development of dark barring on the flanks; the streaks at the side of the breast are concentrated in a restricted area as in *villosus* but individually are intermediate in breadth; the bars of the under tail coverts of *scalaris* are reduced to triangular or sagittate spots. Similar spots result in the *pubescens* × *nuttallii* cross (Ridgway, 1886: 522).

Upper surface. Villosus has a black crown bordered in females by a narrow white bar, connecting anteriorly with the white superciliary stripes; the back and rump are black with a central, longitudinal white stripe extending from the neck to the lower back; medially the white stripe consists of pure white feathers but laterally of feathers with the median vane white and the lateral vane black, these lateral feathers

TABLE 1. *Measurements of females (millimeters and grams)*

Form	No.	Wing length		Bill length from nostril		Tarsal length		Middle toe without claw		Weight	
		M	σ	M	σ	M	σ	M	σ	M	σ
D. villosus	16	123.3	2.9	22.2	1.1	21.0	0.5	13.3	0.6	[1]52.3	4.3
D. scalaris	16	107.2	2.2	16.4	0.7	17.6	0.6	12.0	0.6	[2]31.1	1.3
Hybrid	1	112.3	—	19.5	—	18.6	—	12.0	—	40.2	—
D. borealis	11	115.2	1.3	16.0	0.6	19.9	0.7	13.2	0.7	—	—

[1] 13 measurements only; [2] 15 measurements.

sometimes with black and white on one vane arranged in the form of 2 or 3 partial bars. *Scalaris* has the black of the crown confluent with that of the neck; the rump is black; the back is boldly cross-barred, black and white, both medially and laterally, each feather usually possessing 3 white and 3 black bars of about equal width. In the hybrid the nuchal white collar of *villosus* is represented by a very narrow and broken line of white; the center of the back is cross-barred, coarsely, the bars not aligned as in *scalaris,* thus giving a somewhat spotted effect; no back feathers are pure white and each usually has 2 white bars and 2 black bars; laterally the white is reduced in areas that are black in *villosus* and the feathers here usually have a single white spot or bar terminally but some, especially anteriorly, are pure black. Thus the pattern of the white on individual feathers is dominated by the cross-barring of *scalaris* but the white tends to be concentrated in the mid-back areas as in *villosus.*

Side of head. In *villosus* the narrow white superciliary stripe is bordered below by a wide orbital and auricular black area; a broad white suborbital stripe is connected with white nasal tufts. *Scalaris* has a broad white superciliary stripe that connects ventrally at the posterior end with the suborbital white stripe; consequently the postorbital black area is narrow; the suborbital white stripe touches the orbit anteriorly and connects with brownish nasal tufts. In the hybrid the superciliary white is almost as broad as in *scalaris* but it is only weakly connected posteriorly with the suborbital white stripe; the extent of the black postorbital area is intermediate; the white suborbital stripe touches the orbit but is mottled with black at this point; the nasal tufts are grayish brown almost as in *scalaris.*

Wing. In *villosus* the lesser, middle and greater secondary coverts and the inner secondaries are all black (race *icastus*); the outer primaries and the primary coverts have small white spots (3–5 per feather) on the outer vanes and partial bars of like number on the inner vanes. In *scalaris* all coverts and wing feathers are prominently spotted and barred. In the hybrid the lesser and greater secondary coverts are black as in *villosus;* most of the middle coverts have a single white spot; the inner secondaries show small white spots, not bars, on both vanes and thus have much less white than in *scalaris;* the outer secondaries and primaries and primary coverts are intermediate in amount of white, the parent species not being greatly different in this regard.

Tail. In *villosus* the outer tail feathers, pair 6, are white; pair 5 is white with black spots medially; pair 4 is white laterally with black and white medially in partly barred pattern. In *scalaris* pair 6 has 6 or 7 white crossbars of about equal width; pair 5 is similar but the base of the inner web is black; pair 4 has bars on the outer web and black on the medial web. In the hybrid the outer pair has the outer web white basally with 3 black bars distally, the inner web having 6 black and white bars, the white bars being about twice as broad as the black; pair 5 has 5 broad white and 5 narrow black bars on the outer web and 3 equal black and white bars distally on the inner web, with black basally; pair 4 shows 5 large white spots on the lateral web and the inner web is black. The pattern of barring resembles *scalaris* more than *villosus,* but the greater breadth of the white elements and the white base of the outer vane of pair 6 reflect the influence of *villosus.*

Dimensions. Representative measurements in table 1 show the position of the hybrid with respect to the parental species. The data for *villosus* are based on a combined series of two slightly different size races, *icastus* (6) and *leucothorectis* (10), either of which might have contributed the *villosus* parent. The data on *scalaris* are from the race *cactophilus* of the area concerned and from areas lying to the westward.

The most striking difference between *villosus* and *scalaris* in measurements is in weight which reflects body mass, *villosus* being 70 per cent larger. The hybrid stands approximately at the midpoint between the averages of the parents. Wing length and bill length are likewise not far from the midpoints. Tarsal length is distinctly

below the midpoint in the direction of *scalaris* but probably not to a significant degree. The middle toe is the same as the average for *scalaris* but the difference between the species is slight in this regard, *villosus* ranging as low as 12.3 mm. in the sample represented in the table.

Summary: The characters of the hybrid show the intermediacy expected of an F_1 individual. Dominance is shown in only a few characters. The cross-barred feather patterns of the back and tail of *scalaris* prevail generally, with some modifications, over the uniform black or white of *villosus*. On the other hand, restriction or elimination of the white on the lateral aspects of the back and especially on the wing coverts shows dominance, partial to complete, of the *villosus* characters. Similar dominance effects seem to be indicated in the *nuttallii–pubescens* cross to judge from Ridgway's account (*op. cit.*) where similar parental differences are involved.

Possible Bearing of Hybridization on the Origin of the Species *Dendrocopos borealis*

On first inspection the hybrid from Coahuila shows remarkable resemblance to *Dendrocopos borealis* of the pine areas of the southeastern United States, a species that is sympatric in those areas with *D. villosus*. *Borealis* does not meet *scalaris* to the westward, although the distance separating them in central Texas is not great. The interaction of factors for *villosus* and *scalaris* make in the hybrid a ventral color pattern that is a nearly perfect replica of *borealis;* this duplication extends even to the peculiar triangular spots of the under tail coverts. The broadened and dominant white bars of the lateral rectrices of the hybrid strongly suggest but do not precisely match the same area in *borealis*. Dorsally the pattern of the hybrid departs from *borealis* in the same way that it does from *scalaris,* although the mass effect of reduced white plus the dominance of barring makes the hybrid similar in general appearance to *borealis;* this similarity does not extend, however, to the wing covert markings where *villosus* factors apparently dominate. But in the pattern of the side of the head the hybrid contrasts distinctly with *borealis,* for in the latter the auricular area is white and the small dark postorbital stripe turns dorsally and intercepts the white superciliary stripe. The peculiarly restricted red of the nape of males of *borealis* of course can not be compared; however, this red of *borealis* seems to be merely a reduction to a lateral remnant of the nuchal bar found in males of many species of *Dendrocopos*.

In size *borealis* is much the same as the hybrid (table 1). The bill is disproportionately short and the wing tip is long and the primaries are of different relative lengths as pointed out by Ridgway (1914: 268). However, these are matters subject to considerable racial modification within species of *Dendrocopos*.

The combination of features in the hybrid suggests how a number, if not all, of the characters of the species *borealis* could have been attained through crossing and/or introgression of *villosus* and *scalaris*. In this connection one can not escape the thought, often times expressed elsewhere, that recombinations in secondary contacts may occasionally lead in animals to new and successful gene complexes, which if appropriately isolated or sufficiently superior might set up new species within a generic group. The question that will always remain is how prevalent is such a method of speciation. It should be stressed that we have no proof that this general method was a part of the history of *borealis,* for there are characters in its head markings, especially, that would not seem to be derivable from crossing or through later segregation from such a crossing. These features might, however, have been added in the course of a long subsequent history as a species entity.

The original ladder-backed stock of the genus (Voous, 1947: 94) may have spread across North America south of the boreal floral areas. Its southeastern representation may have met *villosus* stock

there, ranging south, as now, from the boreal forests. Border contact of a limited sort followed by introgression or sympatric hybridization followed by introgression could have provided some of the features of *borealis* now seen in this ladder-backed type. On the contrary, *borealis* and the *villosus–scalaris* hybrid may have merely a fortuitous resemblance resulting from reassortment of comparable or identical genes for size and pattern that are widely present in the genus.

Summary

A natural hybrid between two different species groups of woodpeckers of the genus *Dendrocopos* was taken in Coahuila, México, under circumstances suggesting that the breakdown of biologic isolation of the parental types may have resulted from unavailability of normal mates in one of the species concerned owing to depletion or absence of an effective breeding population in it. A compensatory ecologic extension and prevalence of the other species in the area may have been a contributing factor.

Most of the characters of the hybrid show the intermediacy expected in an F_1 between the parent species *Dendrocopos villosus* and *Dendrocopos scalaris*. Dominance of the cross-barring pattern of *scalaris* and of the reduced wing covert spotting of *villosus* is indicated. Similar interaction of factors is suggested in a cross reported in 1886 between two other species representing the same species groups.

Another species, *borealis*, of the southeastern United States, shows some striking resemblances to the hybrid from Coahuila. A possible history of hybridization and introgression is tentatively suggested for *borealis*.

Acknowledgment

Support of field work in Coahuila was afforded by the Associates in Tropical Biogeography of the University of California. Many courtesies were graciously extended to our field party by Señor Ramon D. Bosquez who has extensive holdings in the Sierra del Carmen of Coahuila. Permission to conduct scientific work in México was kindly granted by the Dirección General Forestal y de Caza of the Mexican government.

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A STUDY OF ALTITUDINAL RACES IN *RANA PIPIENS*[1]

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Received Dec. 15, 1954

INTRODUCTION

Of all the Mexican Amphibia only one species, *Rana pipiens,* is found in all the biotic zones of Mexico. It inhabits the high mountains, the deserts, and the tropical lowlands. The only general factor that limits its distribution is the absence of sufficient water. The overall range of *Rana pipiens* is from north central Canada to Panama, and consequently the unique distribution within Mexico is but a reflection of the adaptability of the species over its entire range.

The work of Moore (1946, 1947 and 1949) has demonstrated that local populations of *Rana pipiens* in Canada, eastern United States, and in lowland Mexico differ from each other with respect to a number of embryonic features. The differences between the populations were demonstrated to be correlated with environmental temperatures and thus of direct adaptive significance. It was further shown that when frogs from different localities are crossed there is an increase in hybrid abnormalities as the north-south distance between the parental populations increases.

Later work by Moore (1950) demonstrated that male *Rana pipiens* from the mountains of Costa Rica, when crossed with females from northeastern United States produced very slight hybrid abnormalities. This was an unexpected result inasmuch as the north-south distance between these two parental populations is greater than that of any other cross. Since only male Costa Rica frogs were available for the experiments no embryological data were obtained.

The work reported here is a study of a number of populations of *Rana pipiens* from Mexico. The populations studied are from the highlands of the Mexican Central Plateau, the lowland tropics, and intermediate altitudes. The purpose of this study was to determine some of the embryonic features that characterize each of these populations, and to study further their genetic structure by means of crossing frogs from the different localities with each other and with frogs from the northeastern United States. By the use of these methods some aspects of the adaptations of this species to the various altitudes may be ascertained, and a picture of the genetic differentiation of each population and of the gene flow between the populations is also provided. By analogy it was believed that this work on Mexican populations of *Rana pipiens* would provide some insight into the paradoxical results obtained with the Costa Rican frogs. Furthermore, it was hoped that this type of data would provide some evidence concerning the historical zoogeography of *Rana pipiens.*

MATERIALS

Frogs collected at the following localities were used in the experiments (also see figure 1).

Zempoala. The Lagunas de Zempoala, Morelos. These are a series of small lakes located in the Sierra de Ajusco, south of Mexico City, at an altitude of approximately 3,000 meters (9,840 feet). The lakes are surrounded by a coniferous forest and form a closed drainage system. Climatological data for Zempoala are not available; however, data that are com-

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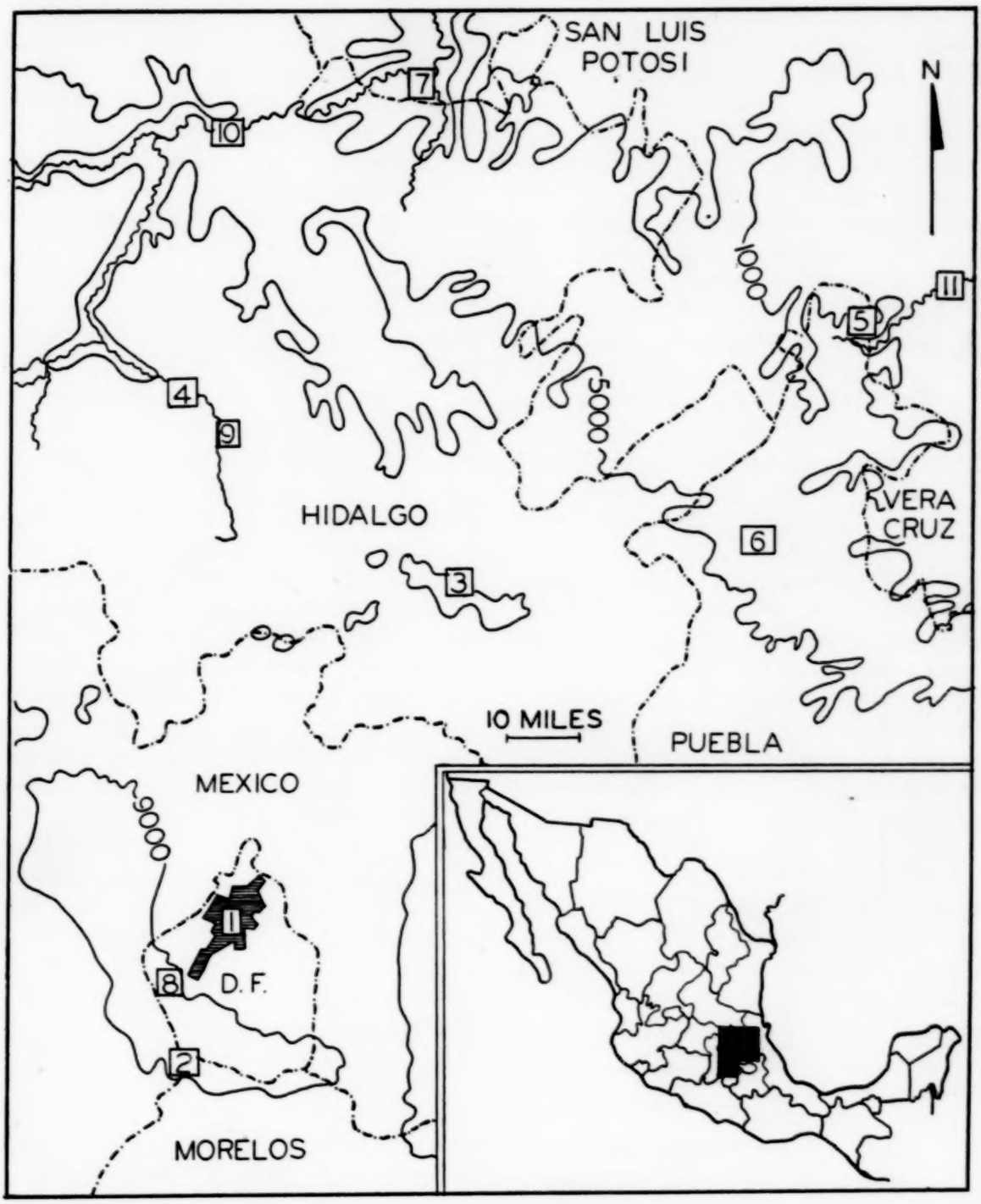

FIG. 1. Map showing the localities where frogs were collected. The map is redrawn from the 1:1,000,000 World Aeronautical Chart, Department of Commerce, U. S. Coast and Geodetic Survey. 1. Mexico City; 2. Lagunas de Zempoala; 3. El Chico; 4. Rio Tula; 5. San Diego; 6. Villa Juarez; 7. Tamazunchale; 8. Desierto de los Leones; 9. Ixmiquilpan; 10. Rio Moctezuma; 11. Rio Cazones. Contour interval 4,000 feet.

parable are available from the Desierto de los Leones, D. F. (Contreras, 1942). This locality is also in the Sierra de Ajusco, 22 kilometers northwest of Zempoala, at an altitude of 3,220 meters (10,500 feet). The mean monthly temperatures for the Desierto de los Leones are plotted in figure 2. It is probable

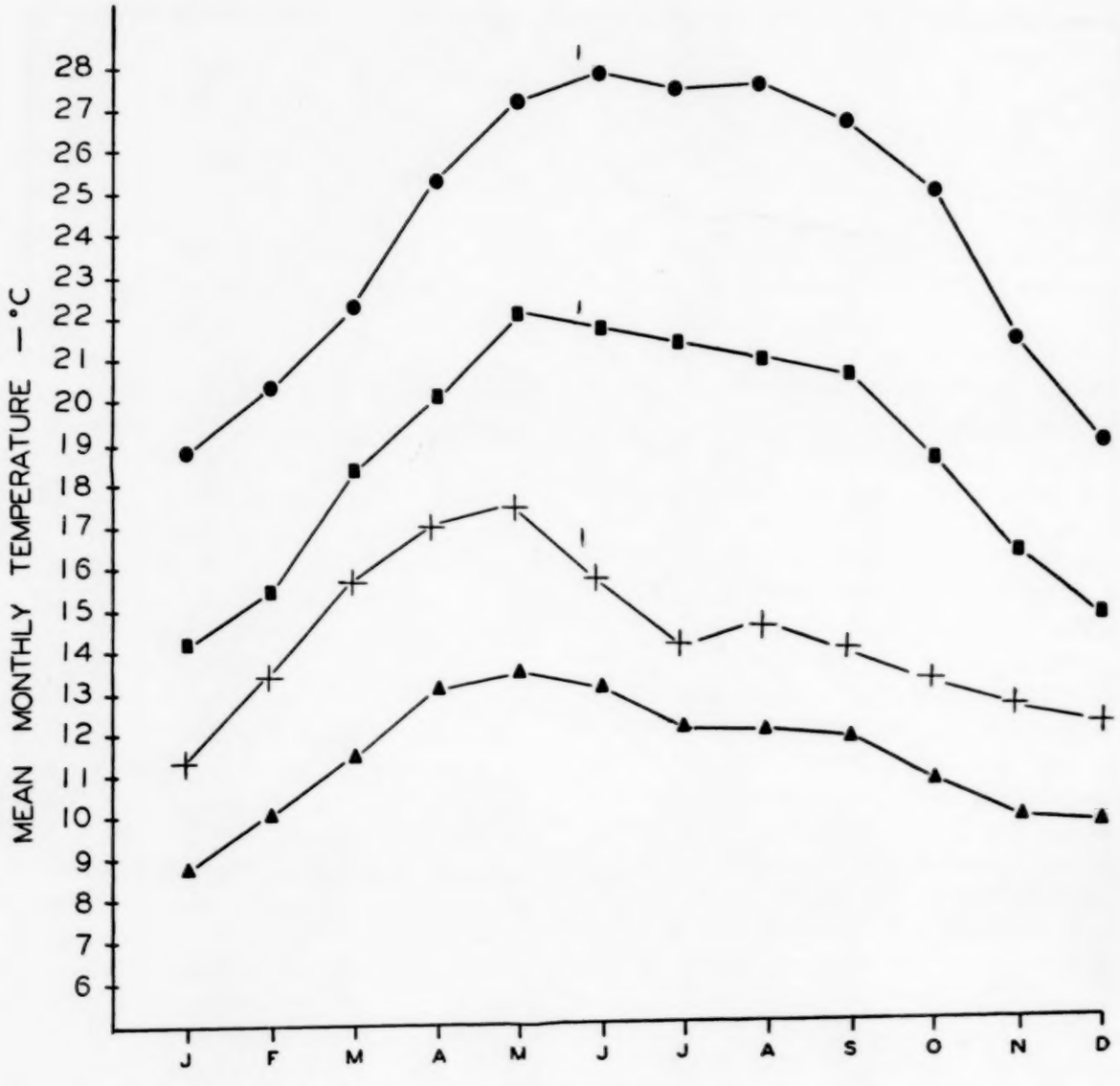

FIG. 2. The mean monthly temperatures of four Mexican localities. See text for further details.

that the mean monthly temperatures at Zempoala are slightly higher than at the Desierto, since Zempoala is about 200 meters lower than the Desierto. Temperatures fall below freezing during the winter months throughout the Sierra de Ajusco.

El Chico. This locality is north of the city of Pachuca, in the Sierra de Pachuca, Hidalgo. The frogs were obtained near the village of Pueblo Nuevo, at the edges of the Parque Nacional el Chico, at an altitude of approximately 2,900 meters (9,500 feet), from two small, permanent pasture ponds. Like Zempoala this locality is characterized by coniferous forest. The region is part of the drainage system of the Rio Moctezuma. Temperature data

(Contreras, *supra cit.*) are available for the town of Mineral el Chico, about 7 kilometers north of Pueblo Nuevo, at an altitude of 2,396 meters (7,850 feet). The mean monthly temperatures for Mineral el Chico are plotted in figure 2. It should be noted that the temperatures near Pueblo Nuevo are probably slightly cooler than at Mineral el Chico, since Pueblo Nuevo is at a higher elevation. The temperature of the water in one of the ponds collected was 16.2° C. at the surface in July. Temperatures fall below freezing during the winter.

Rio Tula. This is at the junction of the Mexico City-Laredo Highway and the Rio Tula, near the town of Tasquillo, Hidalgo. Frogs were collected along the banks of the Rio Tula at an elevation of approximately 1,600 meters (5,250 feet). The Rio Tula drains into the Rio Moctezuma. This is a very arid region supporting a cactus desert flora. Comparable temperature data (Contreras, *supra cit.*) are available from Ixmiquilpan at an altitude of 1,745 meters (5,730 feet), 18 kilometers southeast of the collecting site. The mean monthly temperatures at Ixmiquilpan are plotted in figure 2. Conditions at the Rio Tula site are probably slightly warmer than at Ixmiquilpan. Below freezing temperatures may occur during the winter at Ixmiquilpan.

San Diego. This locality is at kilometer 263 on the Mexico City-Tuxpan Highway, near the village of Maria Andrea, Puebla. Collecting was at an altitude of 350 meters (1,150 feet) on the Mesa de San Diego, part of the Sierra Madre Oriental, in a series of shallow temporary pasture ponds. The top of the Mesa de San Diego is under cultivation; however, the original vegetation of the region was a tropical evergreen forest (Bravo and Ramirez, 1952). These authors give temperature data from Papantla, Veracruz, 38 kilometers east of the Mesa de San Diego, at an altitude of 299 meters (980 feet). The mean monthly temperatures for Papantla are plotted in figure 2, and undoubtedly are very close to those that would be obtained at the Mesa. The water temperature of one of the ponds in August at the collecting site was 26° C.

Villa Juarez. Collecting was on the outskirts of the town of Villa Juarez, Puebla, at an altitude of approximately 1,200 meters (3,935 feet), along the banks of a small permanent stream. The vegetation of this region is of the cloud forest type (Miranda and Sharp, 1950). Climatological data for Villa Juarez or any nearby locality are not available. However, the climate is humid and cooler than that of the Mesa de San Diego. Near freezing temperatures may occur during some winters at this locality.

Tamazunchale. Collecting was along the banks of the Rio Moctezuma, 1.5 kilometers south of Tamazunchale, San Luis Potosi, at an altitude of 208 meters (680 feet). This locality is in the tropical evergreen forest zone, and its climate is comparable to that of Papantla, Veracruz.

Vermont. Frogs were obtained from the Lake Champlain region of northern Vermont from professional dealers at Alburg, Vermont.

Methods

Eggs were obtained by the standard pituitary injection method. They were stripped into sperm suspensions prepared with spring water. When using constant temperature baths, 25 fertilized eggs in clumps of five or less, were placed into a finger bowl. When the eggs were in first cleavage the bowls were immersed in the constant temperature bath. The temperature of the bath was controlled by the use of a mercury thermostat, and the variations in temperature within the finger bowls did not exceed ± .2° C.

When it was desirable to have more eggs than could be accommodated within the constant temperature baths, the additional eggs were placed in a forced draft refrigerator set at approximately 19° C. The eggs treated in this fashion were employed only in determining viability, not developmental rates.

The determination of developmental rates at 12.6 ± .3° C. was not carried out in a constant temperature bath, but rather in a forced draft cold room with the finger bowls of eggs immersed in water.

Rates of development were determined by staging the embryos according to Shumway (1940), with stage 3 (first cleavage) considered as zero time. The end point chosen was the start of gill circulation (stage 20 Early). The time required for more than half of the eggs in a bowl to reach stage 20 E was taken as the final time reading for that experiment. Twenty-five or more eggs were used in each determination of developmental rate.

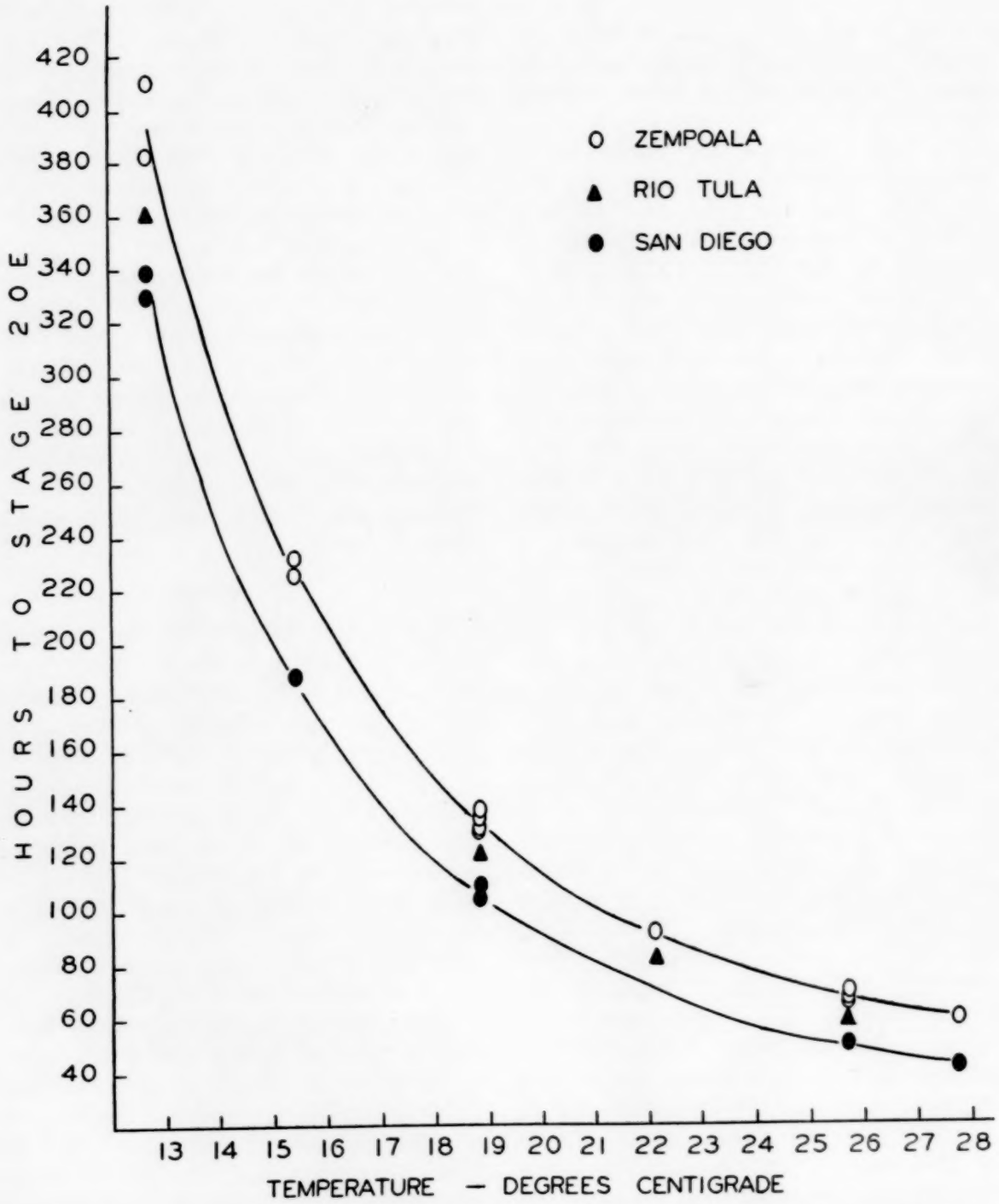

FIG. 3. The rate of development of *Rana pipiens* embryos from three Mexican localities.

TABLE 1. *The number of hours required to reach stage 20 E by Mexican embryos, at different temperatures*

Each cross consists of a different female and a different male frog. Most crosses were tested at more than one temperature.

Crosses		Temperature °C.						
		12.6±.3	15.4±.2	18.8±.2	22.2±.2	25.8±.1	27.8±.1	31.9±.2
Zempoala ♀ × Zempoala ♂	Cross A				94.75			
	Cross B	382				65.5		
	Cross C	409		138		69.5		
	Cross D			129		68		
	Cross E		232.5	136.5				
	Cross F		225	132			58.5	
El Chico ♀ × El Chico ♂	Cross A				99.75			
Rio Tula ♀ × Rio Tula ♂	Cross A				89.25			
	Cross B	361.5		122.5		58.0		
San Diego ♀ × San Diego ♂	Cross A	330				51.75		
	Cross B	339				51		
	Cross C		187.5	106			44	
	Cross D							41.5
	Cross E			110				

Crosses between frogs from the different Mexican localities and with Vermont *Rana pipiens* were also carried out. When conducting hybrid crosses a parallel control (eggs of the same female and sperm of a male from the same locality as the female) was always run. The rates of development for the hybrid crosses could then be compared to the rate of the control cross. During stage 20–21 the number of embryos that were dead (cytolyzed or partially cytolyzed, and without muscular response to direct physical stimulation) were counted. This constitutes a measure of the viability of the crosses, and is represented in the data as the per cent mortality. Actually the per cent mortality reported for the different test crosses have been corrected by taking into consideration the mortality of the parallel controls.[1] A total of 28 parallel controls were run in determining the per cent mortality. Of these controls sixteen had 0 per cent mortality, seven had a mortality of less than 11 per cent, four controls had a mortality between 12 per cent and 20 per cent, and a single control had a mortality of 32 per cent. In all cases the observed mortality of the test cross was greater than the mortality of the parallel control.

Some tadpoles were raised to metamorphosis. The tadpoles were reared in large finger bowls, and fed a mixed diet of egg yolk, pablum and spinach.

RESULTS

Rates of development. In table 1 the developmental rates of embryos of *Rana pipiens* from four different localities and at different temperatures are tabulated. The same data for Zempoala, Rio Tula, and San Diego are plotted in figure 3.

These data very clearly show that the frogs from the high altitude localities of Zempoala and El Chico produce embryos that have a very slow developmental rate. In the case of El Chico embryos this has

[1] The correction is made in the following manner:

$$\frac{T - C}{1 - C} = \%\ \text{mortality},$$

where T is the observed per cent mortality of the test cross and C is the observed per cent mortality of the parallel control.

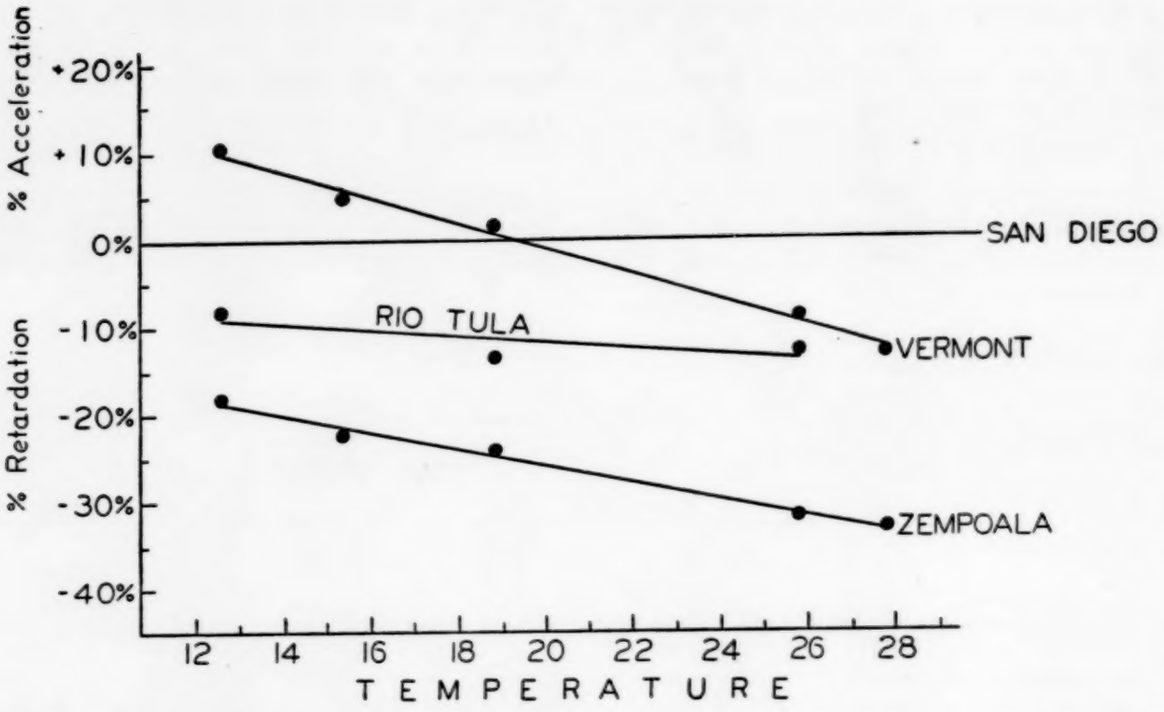

FIG. 4. Comparison of rates of development of embryos of *Rana pipiens* from three Mexican localities and Vermont. The mean number of hours from stage 3 to stage 20 E was used in making the comparison. See text for further explanation.

only been determined at a single temperature. In comparison the lowland tropical frogs from San Diego have a very rapid developmental rate. The embryos from Rio Tula, an intermediate locality in regard to altitude and mean monthly temperature, have an intermediate developmental rate. They are faster than Zempoala embryos but slower than San Diego eggs.

The differences in developmental rates between San Diego *pipiens* and Zempoala and Rio Tula *pipiens* can also be demonstrated as per cent retardation in rates at the different temperatures.[2] In figure 4 the rates of Zempoala, Rio Tula, and Vermont embryos are compared to the rate of the San Diego embryos. The per cent retardation of Zempoala embryos, as compared with San Diego, increases with temperature. Thus at 12.6° C. the Zempoala embryos show an 18 per cent retardation, while at 27.8° C. they are retarded by 33 per cent. In the case of the Rio Tula embryos there is only a slight increase in the retardation, from 8 per cent at 12.6° C. to 13 per cent at 25.8° C. The relation of the developmental rates of Vermont embryos to the San Diego base line shows that Vermont has a more rapid development at temperatures below 19.5° C., but has a slower development than San Diego at temperatures above 19.5° C. The relation of the San Diego and Vermont rates of development is typical of all comparisons of northern and southern population of *Rana pipiens* (see Moore, 1949).

In figure 5 the same comparison is made, using Vermont as the zero per cent base line, and including the slopes of some United States populations of *Rana pipiens* (data for United States populations from Moore, 1949). It is clear from figure 5 that the developmental rates of

[2] The per cent retardation or acceleration is measured at a given temperature by $T_1 - T_2/T_1 \times 100$. In figure 4 T_1 was the mean number of hours required for San Diego embryos to reach stage 20 E. In figure 5 T_1 was the time required for Vermont embryos to reach the same stage. T_2 is the time required by embryos from the locality being compared to reach stage 20 E at the same given temperature.

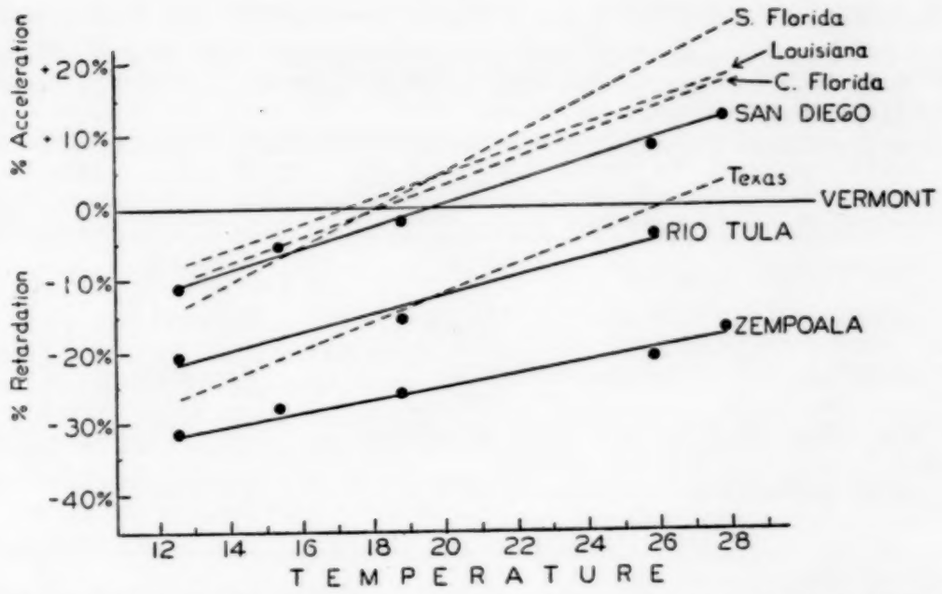

FIG. 5. Comparison of rates of development of embryos of *Rana pipiens* from Mexican and United States localities. The mean number of hours from stage 3 to stage 20 E was used in making the comparison. Data for southern Florida, central Florida, Louisiana, and Texas embryos were obtained from Moore (1949). See text for further explanations.

Zempoala embryos are slower at all temperatures than those obtained for any other population of *Rana pipiens*.

Hybrid crosses. Table 2 presents a tabulation of some of the results of the crosses involving Mexican eggs. Table 3 is a similar tabulation of the results of crosses, using Vermont eggs. The column headed "% Retardation (−) or Acceleration (+) to Stage 20E," represents the result of comparing the rate of development of the hybrid embryos with the average rate of development of embryos from the same locality as the eggs used in the hybrid cross. The developmental rates at 18.8° C. were used in making this comparison, except in cases of Tula ♀ × Tamazunchale ♂ and Zempoala ♀ × Tula ♂

TABLE 2. *Results of hybrid crosses using Mexican females*

The figures in the column marked "Number of crosses" represent the number of different pairs of frogs used. Some frogs were used in more than one cross.

	No. of crosses	Rate of development	% Retardation (−) or % acceleration (+) of developmental rate	Head size	% Mortality
Zempoala ♀ × El Chico ♂	1	Normal	0	Normal	0
Zempoala ♀ × Rio Tula ♂	1	Normal	+2	Normal	0
Zempoala ♀ × San Diego ♂	4	Accelerated	+8 (approx.)	Slightly enlarged	0
Zempoala ♀ × Vermont ♂	3	Normal	0	Reduced	0
El Chico ♀ × San Diego ♂	1	Normal	0	Slightly enlarged	14
Rio Tula ♀ × Zempoala ♂	1	Normal	0	Normal	8
Rio Tula ♀ × San Diego ♂	1	Accelerated	+6	Normal	2
Rio Tula ♀ × Tamazunchale ♂	1	Normal	+2	Normal	0
Rio Tula ♀ × Vermont ♂	2	Retarded	−7	Very reduced	0 and 8
San Diego ♀ × Zempoala ♂	2	Retarded	−17	Slightly reduced	0
San Diego ♀ × El Chico ♂	2	Retarded	−14	Slightly reduced	0
San Diego ♀ × Rio Tula ♂	2	Retarded	−7	Normal	0
San Diego ♀ × Villa Juarez ♂	2	Retarded	−5	Normal	0
San Diego ♀ × Vermont ♂	2	Retarded	−40 (approx.)	Extremely reduced	0 and 72

TABLE 3. *The results of hybrid crosses between Vermont females and Mexican males*

The figures in the column marked "Number of crosses" represent the number of different pairs of frogs used. Some frogs were used in more than one cross. The mean value of % retardation and % mortality are in parentheses below the extreme values.

	No. of crosses	% Retardation in developmental rate	Head size	% Mortality to stage 20
Vermont ♀ × Zempoala ♂	3	15–17	Enlarged	6–31 (20)
Vermont ♀ × El Chico ♂	10	13–22 (17)	Enlarged	2–29 (16)
Vermont ♀ × Rio Tula ♂	9	15–30 (19)	Very enlarged	5–52 (22)
Vermont ♀ × San Diego ♂	12	40 (approx.)	Very enlarged	43–100 (82)
Vermont ♀ × Villa Juarez ♂	5	40 (approx.)	Very enlarged	67–100 (93)

when the developmental rates at 22.2° C. were used.

Slight differences in developmental rates may be obtained when eggs from a single female are fertilized by sperm from two males (from the same locality as the female), and the rates of the two groups of embryos are compared. Consequently differences in the developmental rates of two per cent or less between the hybrids and controls are not considered significant. Similarly, differences in per cent mortality of about five per cent are not considered significant.

Zempoala ♀ × El Chico ♂

A single cross of frogs from the two different high altitude localities was conducted at 18.8° C. and 25.8° C., and the rate of development, as well as all other aspects of development, was identical to the control Zempoala ♀ × Zempoala ♂ cross. Stage 20 E was reached in 129 hours at 18.8° C. and in 68 hours at 25.8° C. Some tadpoles were raised and metamorphosed normally.

Zempoala ♀ × Rio Tula ♂

A single cross of highland females and intermediate males was made at 22.2° C. The cross is perfectly normal in all respects. The rate of development was slightly accelerated (two per cent) as compared to the Zempoala ♀ × Zempoala ♂.

Zempoala ♀ × San Diego ♂

Four separate crosses of highland females with lowland males were made and run at 18.8° C. and at 22.2° C. At both temperatures the rate of development is slightly accelerated (about six per cent at both temperatures). The embryos of this cross are also distinguished by having the head region slightly enlarged when compared to Zempoala ♀ × Zempoala ♂ embryos. In all other respects these crosses were normal.

Zempoala ♀ × Vermont ♂

Three crosses of Mexican highland females and Vermont males were made and conducted at 18.8° C., 22.2° C., and 25.8° C. In all crosses the heads of the embryos were reduced in size, although no other gross abnormalities were evident. The developmental rate is normal; at 22.2° C. the rate is accelerated slightly (approximately four per cent), while one cross at 18.8° C. was accelerated about one per cent, and another at 18.8° C. had the same developmental rate as the control Zempoala ♀ × Zempoala ♂ embryos. The single cross at 25.8° C. when compared to the control was retarded about two per cent. Some tadpoles were raised and metamorphosed normally.

El Chico ♀ × San Diego ♂

A single cross of a highland El Chico female and a lowland male was conducted at 22.2° C. The development of these embryos was irregular. Between stages 14 and 19 the development was slower than in the El Chico ♀ × El Chico ♂ cross, however, stage 20 was reached at the same time as the El Chico ♀ × El Chico ♂ embryos. All embryos were characterized by enlarged heads as in the case of the Zempoala ♀ × San Diego ♂ embryos. There was an estimated 14 per cent mortality.

Rio Tula ♀ × San Diego ♂

A single cross of an intermediate Rio Tula female and a lowland male was conducted at

18.8° C. and 25.8° C. At 25.8° C. there was an acceleration of three per cent in the developmental rate. At 18.8° C. the developmental rate was accelerated by six per cent, and at stage 20 there was a mortality of two per cent. In all other respects the cross was normal. Some tadpoles were raised and metamorphosed normally.

Rio Tula ♀ × Zempoala ♂

A single cross of an intermediate Rio Tula female and a highland male was conducted at 18.8° C. The rate of development was identical to the Rio Tula ♀ × Rio Tula ♂ cross, and development appeared to be normal in all respects. However, at stage 20 there was an eight per cent mortality. Some tadpoles were raised and metamorphosed normally.

Rio Tula ♀ × Tamazunchale ♂

A single cross of an intermediate Rio Tula female and lowland Tamazunchale male was conducted at 22.2° C. As in the case of the Rio Tula ♀ × San Diego ♂ cross there was a slight acceleration of the developmental rate (two per cent). Development was normal in all other respects and no mortalities occurred by stage 20. Tadpoles were raised and developed normally until aged 42 days, at which time the experiment was terminated.

Rio Tula ♀ × Vermont ♂

Two crosses of Mexican female frogs from an intermediate altitude with Vermont males were made, one at 18.8° C. and another at 22.2° C. In both cases the developmental rate was retarded between five per cent and 10 per cent. The retardation is first evident during gastrulation. Although the yolk plug appears normal in size, gastrulation is retarded. Furthermore, when the hybrid embryos were at stage 12L the control embryos were at stage 13. During stage 12L the hybrid embryos were characterized by a persistent blastocoel, that appears externally as an anterior-ventral constriction of the embryo. This constriction remains as the swollen abdomen of later stages. The head of these embryos is reduced, more so than in the Zempoala ♀ × Vermont ♂ embryos. A mortality of eight per cent was found in the cross run at 18.8° C. Twenty-eight tadpoles of this cross were raised. Ten were normal in coloration and metamorphosed. However, 17 failed to metamorphose and showed no limb buds when aged 141 days (23 days after the last normal colored tadpole had metamorphosed). Of the tadpoles that did not metamorphose 15 were albino and the rest normal in color.

San Diego ♀ × Zempoala ♂

Two crosses of lowland females with highland males were made at 18.8° C. The rate of development was retarded by 17 per cent. Development was normal in all other respects except for the fact that the embryos were characterized by slightly reduced heads. Some tadpoles were raised and metamorphosed normally.

San Diego ♀ × El Chico ♂

Two crosses of lowland females with highland El Chico males were made, one at 18.8° C. and the other at 22.2° C. The developmental rate was retarded approximately seven per cent at 22.2° C. and 14 per cent at 18.8° C. Like the San Diego ♀ × Zempoala ♂ embryos these embryos were characterized by having reduced heads, but they were normal in all other gross aspects.

San Diego ♀ × Rio Tula ♂

Two crosses of lowland females with intermediate Rio Tula males were made, one at 18.8° C. and another at 18.8°, 12.7°, and 25.8° C. In both crosses, and at all the temperatures tested, the developmental rate was delayed between five per cent and eight per cent. Development was normal in all other aspects.

San Diego ♀ × Villa Juarez ♂

A single cross was made at 18.8° C. There was a slight retardation in development of about five per cent. The embryos were normal in all other respects.

San Diego ♀ × Vermont ♂

Two crosses of lowland Mexican females and Vermont males were made at 18.8° C. This cross produces extremely abnormal embryos. By stage 12 retardation in development is evident and a persistent blastocoel, covered by ectoderm, protrudes from the ventral region of the embryo. From this time on the embryos are very difficult to stage due to grossly abnormal development. There is a persistent blastocoel and the abdomen is distended in all embryos after stage 12. The head is extremely reduced, more so than in the Rio Tula ♀ × Vermont ♂ hybrids. In one of the crosses made none of the embryos died before the majority of them had reached stage 20, however, by the sixteenth day all were dead, and none had been able to start feeding. In the other cross there was a 72 per cent mortality by stage 20, and by the fourteenth day only three albino embryos of a total of 200 fertilized eggs remained alive. The same three embryos were still alive 87 days after fertilization. The developmental rate in these crosses was estimated to be retarded about 40 per cent. This is a very approximate estimate, however, since the extreme abnormalities of these embryos makes comparison difficult.

Vermont ♀ × Zempoala ♂

Three crosses of Vermont females and highland Mexican males were conducted at 18.8° C. In contrast to the reciprocal cross, this cross produces embryos that are markedly delayed in development. The retardation is between 14 per cent and 16 per cent. The mortality in the three crosses was six per cent, 22 per cent, and 31 per cent. The head of the embryos is enlarged and the body is slightly shortened in comparison to the control embryos. The yolk plug is slightly enlarged in some of the embryos. Some tadpoles were raised and these metamorphosed normally, inasmuch as the defects were restricted to the early stages.

Vermont ♀ × El Chico ♂

Ten crosses of Vermont females and highland El Chico males were made at 18.8° C. The morphological characters of the embryos of this cross are identical to those obtained in the Vermont ♀ × Zempoala of embryos.[3] The developmental rate was retarded from 13 per cent to 22 per cent, and the mortality for the different experiments was two per cent, three per cent, four per cent, six per cent, 12 per cent, 19 per cent, 19 per cent, 25 per cent, 31 per cent, and 34 per cent. Some tadpoles were raised and these metamorphosed normally.

Vermont ♀ × Rio Tula ♂

A total of nine crosses of Vermont females and Mexican intermediate males were made at 18.8° C. Some of the embryos are characterized by having a markedly enlarged yolk plug, more enlarged than in the Vermont ♀ × Zempoala ♀ and Vermont ♀ × El Chico ♂ embryos. The head of these embryos is also more enlarged and the body more shortened than in the case of Vermont ♀ × Zempoala ♂ and Vermont ♀ × El Chico ♂ embryos. Heart beat appears to be delayed and when present is in many cases abnormally pronounced. Gill circulation is often abnormal. The retardation in development is from 14 per cent to 29 per cent, and the mortality was 5 per cent, 9 per cent, 9 per cent, 10 per cent, 11 per cent, 20 per cent, 29 per cent, 49 per cent, and 52 per cent. Some tadpoles were raised and these metamorphosed normally.

Vermont ♀ × San Diego ♂

A total of 12 crosses of Vermont females with lowland Mexican males were conducted at 18.8° C. The yolk plug of these embryos is even more enlarged than in the other crosses, and customarily the blastopore is never closed. The embryos died at what would correspond to stage 15. The embryos that survive past neurula have very shortened bodies and enlarged heads. Heart beat is very delayed and very pronounced when it does appear. Gill circulation is abnormal. The retardation in the developmental rate cannot be estimated accurately because of the marked abnormalities of the embryos. Retardation is probably greater than 40 per cent. The mortality for the different experiments was 43 per cent, 50 per cent, 72 per cent, 76 per cent, 79 per cent, 90 per cent, 91 per cent, 93 per cent, 97 per cent, 99 per cent, 100 per cent, and 100 per cent. Of the total of 1,570 fertilized eggs produced in the 10 crosses only 8 tadpoles lived to metamorphose.

Vermont ♀ × Villa Juarez ♂

A total of five crosses at 18.8° C. were made. Morphologically the development of these hybrids is identical to that of Vermont ♀ × San Diego ♂. The mortality for the different experiments was 67 per cent, 98 per cent, 99 per cent, 100 per cent, and 100 per cent.

Adult morphology. The specimens of *Rana pipiens* from the different Mexican localities differ markedly from each other in their adult morphology. The frogs from El Chico and Zempoala are virtually identical and individuals are impossible to tell apart. These high altitude frogs are characterized by having a green ground color on the dorsum, having yellow pigment on the ventral surface (especially on the legs), and short blunt snouts. The males are without vestigial oviducts. The largest specimen, of more than 100 specimens from El Chico and Zempoala, is a female having a snout-to-vent length of 66 mm. The external morphology of El Chico and Zempoala frogs resembles that of Vermont frogs more than it does the morphology of lowland San Diego frogs.

In contrast the specimens from San Diego are characterized by having a brown ground color to the dorsum, being devoid of any trace of yellow pigment on the ventral surface, and having a long snout. All males were found to have vestigial oviducts. Of a total of 60 specimens, the maximum snout-to-vent length found was a female measuring 101 mm. The frogs

[3] I have examined preserved stage 20 embryos of the Vermont ♀ × Costa Rica ♂ crosses made by Moore (1950). The morphology of these embryos is the same as that of the embryos from Vermont ♀ × Zempoala ♂ and Vermont ♀ × El Chico ♂ crosses.

from Villa Juarez and Tamazunchale are very similar to the San Diego specimens.

The Rio Tula frogs are intermediate in their morphology when compared to the high altitude frogs and to the low altitude frogs. The ground color of the dorsum is usually of a greyish color, the ventral surface of the legs may or may not have some yellow pigment, and the snout shape is similar to that of the Zempoala and El Chico frogs but is not as blunt. Of a total of 18 males only five had vestigial oviducts. The maximum snout-to-vent length from a total of 29 specimens was 86 mm. in a female.

Egg size. The diameters of 30 eggs from two El Chico females ranged from 1.79–2.09 mm., with a mean of 1.92 mm. ($\sigma = .070$ and $\sigma_m = .013$). Ninety eggs from three Zempoala females ranged from 1.72–2.00, with a mean of 1.86 mm. ($\sigma = .077$ and $\sigma_m = .008$). Sixty eggs from three San Diego females had a range of 1.67–1.95 mm., and a mean of 1.84 mm. ($\sigma = .065$ and $\sigma_m = .008$).

Discussion

Moore (1949) demonstrated that the rates of development of *R. pipiens* embryos from northern localities (Vermont, New Jersey, Wisconsin, and Quebec, Canada) were essentially the same. In contrast the rates for embryos from Louisiana, Florida, Texas, and eastern Mexico differed from each other and from the rates of the northern embryos. However, when the developmental rates of northern embryos are compared with those of southern embryos there is a characteristic relationship between the rates (see figure 5); at low temperatures the northern forms are characterized by a more rapid rate than the southern forms, while at higher temperatures the southern forms have a more rapid rate of development. This difference between the northern and southern forms is correlated with the environmental temperatures of the different localities during the breeding season, and can thus be interpreted as evidence of adaptation for a rapid developmental rate at the different environmental temperatures. The rates of development obtained for lowland San Diego embryos are the same as those obtained by Moore (*supra cit.*) for embryos from Axtla, San Luis Potosi, and more recently by Volpe (1954) for embryos from the southern part of the state of Tamaulipas. The developmental rates of all the lowland localities from eastern Mexico are faster than Vermont embryos at temperatures above 20° C.

In contrast to the lowland Mexican forms, the embryos from Zempoala have a slower development rate at all temperatures than any other population of *Rana pipiens*. In other words, the embryos from this high altitude locality do not show any adaptation for a rapid rate of development. The environmental temperatures to which the Zempoala frogs are exposed are far lower than those encountered by San Diego frogs (see figure 2), and consequently one would be led to expect that the developmental rates of Zempoala and San Diego embryos would show the same relation to each other as the rates of northern and southern embryos. Actually the only temperature effect evident is that the per cent retardation of Zempoala embryos is greater at high temperatures (temperatures at which San Diego embryos have a rapid developmental rate) than at low temperatures. The Zempoala embryos can be considered adapted to low environmental temperatures (since they successfully inhabit a region of relatively low temperatures). However, this adaptation has not been associated with a further adaptation for a rapid rate of development at these temperatures. It can, therefore, be suggested that there is no selection for a rapid developmental rate at the high altitude localities, while in the lowlands there is selection for rapid development.

In support of this theory it can be pointed out that the lowland *R. pipiens* are probably subject to more interspecific competition than the highland forms. At

San Diego, in the same ponds in which *R. pipiens* was collected, the following amphibians were found to breed: *Bufo valliceps, Bufo marinus, Rana catesbeiana,* four species of *Hyla,* and *Diemictylus kallerti.* In contrast, the only other amphibians found breeding at the El Chico ponds were *Hyla lafrentzi* and *Ambystoma tigrinum,* and in the Lagunas de Zempoala *Hyla lafrentzi* and *Rhyacosiredon altamirani.* At room temperature and under the laboratory conditions of light and food the first San Diego tadpoles to complete metamorphosis were seventy-three days old. In contrast the first Zempoala tadpoles (raised during the same time and under the same conditions) to complete metamorphosis were ninety-nine days old. Thus a rapid rate of development may be of advantage in removing the frogs from intense interspecific (and possibly also intraspecific) competition in the breeding ponds.

The results obtained from the hybrid crosses provide considerable information regarding the genetic structure of the different populations. The single cross of Zempoala ♀ × El Chico ♂ was normal and suggests that there is little genetic difference between the two highland populations. This is supported by the single determination of developmental rate made of El Chico ♀ × El Chico ♂ embryos, by the fact that the Vermont ♀ × El Chico ♂ crosses give results similar to those of Vermont ♀ × Zempoala ♂ crosses, and furthermore by the extremely similar morphology of the El Chico and Zempoala frogs.

In comparison the crosses involving highland (El Chico and Zempoala) frogs and lowland San Diego frogs are not normal. The morphology of the embryos as well as the developmental rates are affected, and in the El Chico ♀ × San Diego ♂ cross some of the embryos died during early development. Considering the differences in environment between the highland and lowland localities, and the extreme differences in morphology between frogs from these localities, the hybrid abnormalities are less than would be expected.

The crosses involving frogs from the Rio Tula and the highland and lowland frogs are all normal, although there is some modification of rates of development. Thus the Rio Tula population bridges the genetic gap between the two altitudinal extremes. The Rio Tula frogs are an ideal intermediate population since they are intermediate in all the biological aspects considered, and also inhabit a region that is intermediate in respect to altitude and temperature. It is possible that this intermediate condition of the Rio Tula frogs may be the result of "intermediate selection." It should be emphasized, however, that the populations of *R. pipiens* are continuous between the highland and the lowlands inasmuch as the frogs inhabit the lengths of the rivers and streams. Consequently selection may be operating in a clinal manner from the lowlands to the highlands, yet the genetic structure of the populations is also being affected by the gene flow between the populations. Both of these factors are therefore responsible for the characteristics of the Rio Tula population. Furthermore, gene flow may account for the relative normality of the highland × lowland hybrids. It may be considered subjective for the author to state that the highland × lowland hybrids have unexpectedly slight abnormalities. However, it should be noted that the highland Zempoala and lowland San Diego frogs are as different morphologically as Vermont *pipiens* are from Florida *pipiens.*

Further evidence of altitudinal differentiation may be found in the San Diego ♀ × Villa Juarez ♂ cross. The Villa Juarez frogs are sufficiently different genetically to retard the development of the hybrids, although morphologically they are very similar to San Diego frogs, and are located in the same drainage system, only 35 kilometers from San Diego.

Moore (1946, 1949) has demonstrated that *Rana pipiens* is a genetically plastic species that has produced numerous races

in eastern North America in response to the different regional environments. Likewise under the different selective pressures that exist from the tropical lowlands of Mexico to the cool boreal highlands this species has produced locally adapted forms. These forms differ in their morphology and physiology. However, since only a single intermediate population has been investigated in the present study, the species appears to have produced three distinct races in adapting to the range of environments. This is doubtless illusory, and the result of not having investigated further populations from other intermediate localities.

Extensive work has been done with altitudinal races of many plants in California. Clausen, Keck, and Hiesey (1941) have demonstrated that the plant, *Potentilla glandulosa,* which is found from the California coast to high in the Sierra Nevadas, is composed of a number of altitudinal races. These races differ in morphology and physiology from each other and represent adaptations to specific climatic environments. Four major races can be distinguished morphologically, and where they overlap they remain distinct, although there is some interbreeding (Clausen, Keck, and Hiesey, 1947). In the case of *Rana pipiens* the points of junction of the populations studied have still to be determined. However, considering the mobility of frogs in comparison to plants, it is believed that no sharp boundaries will be found between the different forms as is the case in *P. glandulosa.* There probably exists a morphological and physiological spectrum between the two extreme forms of *R. pipiens.* Even in *Potentilla glandulosa* one of the subspecies (*nevadensis*) actually consists of two climatic races and resembles *R. pipiens* in that the two races form an intergrading morphological series. Similarly, in the case of *Achillea lanulosa* (Hiesey, Clausen and Keck, 1942, and Clausen, Keck, and Hiesey, 1948) the climatic races are connected by intergrading populations and thus are not sharply separated as are the races of *Potentilla glandulosa.*

It has been demonstrated by Moore (1946) that as the north-south distance between populations of *Rana pipiens* increases, the abnormalities and the inviability of the hybrids produced between members of these poulations, also increases. This has been interpreted as the result of increasingly different temperature adaptations, and the hybrid abnormalities are consequently the result of incompatibility between the different temperature genomes. The results here obtained from the crosses between Mexican populations and Vermont frogs support this thesis and also give further evidence of the genetic differentiation of the Mexican populations. Both San Diego and Villa Juarez frogs produce extremely abnormal and inviable hybrids when crossed with Vermont frogs. Moore (1947) had previously obtained identical results with frogs from tropical San Luis Potosi. The Rio Tula frogs produce considerably abnormal hybrids when crossed with Vermont frogs, although the embryos are less abnormal than the hybrids of Vermont and lowland Mexican frogs. El Chico and Zempoala frogs produce the least abnormal hybrids when crossed with Vermont frogs. Thus the high altitude, low temperature adapted populations are more compatible with the Vermont low temperature genome than the lowland Mexican forms. Comparable results had been obtained by Moore (1950) when he crossed Costa Rican male *R. pipiens,* obtained at an altitude of 3,700 feet, with female Vermont frogs. This was the first demonstration that hybrid abnormalities did not increase with the north-south distance of the parental populations in so far as highland populations of *R. pipiens* were concerned.

The morphological abnormalities obtained in hybrids of southern and northern populations of *R. pipiens* were first described by Porter (1941, 1942) and Moore (1941, 1946). When an egg from a northern population is fertilized by a

sperm from a southern population a characteristic embryonic syndrome is produced in which the main effect is an enlargement of the embryo's head region. When the reciprocal cross is made the syndrome is characterized by a reduction of the embryo's head region. The degree of expression of these syndromes increases as the north-south distance between the parental populations increases, except in the case of crosses between highland and Vermont frogs. It is therefore of interest to find the same syndrome exhibited by the hybrids of highland and lowland Mexican populations. Thus the highland eggs (Zempoala and El Chico) when fertilized by lowland San Diego sperm produce embryos that have slightly enlarged heads, while San Diego eggs fertilized by highland sperm produce embryos with slightly reduced heads. In this case the syndromes are again correlated with a difference in the environmental temperature of the parental populations. However, it is not in this instance correlated with an adaptation for rapid developmental rates, as was the case in the previous demonstrations of these syndromes.

The expression of these syndromes by the hybrids of Mexican and Vermont *pipiens* varies, depending on the Mexican population that is crossed with Vermont. Thus the heads of the Zempoala ♀ × Vermont ♂ embryos are less reduced than in the case of the Rio Tula ♀ × Vermont ♂ embryos, which in turn have less reduced heads than the San Diego ♀ × Vermont ♂ embryos. In the reciprocal crosses the head is most enlarged in the Vermont ♀ × San Diego ♂ and Vermont ♀ × Rio Tula ♂ embryos, and least enlarged in the Vermont ♀ × El Chico ♂ and Vermont ♀ × Zempoala ♂ embryos. The differences in the degree of expression of the syndromes is consequently directly correlated to the environmental temperatures of the parental population, i.e., the hybrids of Mexican highland and Vermont *pipiens* show less expression of the syndromes than the hybrids of Mexican lowland and Vermont *pipiens*.

When all the hybrid crosses are compared as to degree of normality or abnormality, it is clear that the hybrids between the different Mexican populations are relatively normal in comparison to the hybrids between Mexican and Vermont populations. The only cross between Mexican populations that shows evidence of some abnormalities is that between highland and lowland frogs. The least abnormal of the crosses between Mexican and Vermont populations is that of highland and Vermont frogs. Yet it should be noted that although the embryos of the cross highland ♀ × Vermont ♂ are essentially as normal as the highland ♀ × lowland ♂ embryos, the reciprocal cross, Vermont ♀ × highland ♂, is abnormal in comparison to lowland ♀ × highland ♂ embryos.

In conclusion, the data derived from the hybridization experiments demonstrate that the highland Mexican genotypes are relatively compatible with lowland Mexican genotypes, and that in comparison the highland genotypes are relatively less compatible with the genotype of Vermont frogs. Therefore, even though the Zempoala and El Chico populations are low temperature adapted and are morphologically more similar to Vermont frogs than to lowland San Diego frogs, they are yet genetically more closely related to the lowland Mexican populations. The hybridization experiments have thus provided a better measure of the genetic relations of the different populations than was possible by direct morphological comparison.

Furthermore, the relative compatibility of Mexican highland genotypes with Vermont genotypes, in comparison to the extreme incompatibility of Mexican lowland genotypes and Vermont genotypes, can be attributed to the low temperature adaptation of the highland frogs. A similar explanation was postulated by Moore (1950) to explain the results obtained when Costa Rican males were crossed to Vermont females.

The Mexican highland populations may thus be considered morphologically con-

vergent to the Vermont forms, and to have evolved from adjacent lowland populations. A similar explanation is probably applicable to the Costa Rican population of Moore. Certainly the close relationship, as indicated by the cross fertilization experiments, of the lowland and highland Mexican populations argues against the theory that the highland populations are the result of southern migration of cold-adapted forms from the Rocky Mountains. From the embryological data obtained for the highland populations of Mexican *R. pipiens,* and from the similarity of the Vermont ♀ × Costa Rica ♂ hybrids and the Vermont ♀ × Mexican highlands ♂ hybrids, it could be assumed that the highlands of Costa Rica have populations comparable to the highland Mexican populations. The presence of such low temperature adapted populations of *Rana pipiens* south of the Isthmus of Tehuantepec would also support a theory of independent origin from adjacent lowland populations. The Isthmus has been a region of low relief (and consequently of warm climate) at least since the Miocene (Schuchert, 1935), and would have served as a barrier to the migration of cold-adapted forms. Low temperature adapted, highland populations of *R. pipiens* may therefore exist throughout the mountains of Mexico and Central America, yet these populations may have evolved independently and may be isolated from other highland populations.

Summary and Conclusions

1. The developmental rates of embryos of *Rana pipiens* from three different populations in Mexico were determined. The embryos were from San Diego, a lowland tropical locality, the Rio Tula at an altitude of 1,600 meters, and Zempoala at an altitude of 3,000 meters. The San Diego embryos are characterized by the more rapid rate of development of the three localities at all the temperatures tested. The Zempoala embryos have the slowest rate of development of the three populations, while the Rio Tula embryos have an intermediate rate of development.

2. Hybrid crosses were made using frogs from the above mentioned three localities as well as frogs from El Chico, at 2,900 meters, Tamazunchale, at 208 meters, and Villa Juarez, at 1,200 meters. Crosses between highland and lowland frogs produced embryos that were slightly abnormal in development. Frogs from the intermediate locality of Rio Tula when crossed with either the highland or lowland frogs produced normal hybrids.

3. Further hybrid crosses were made using Vermont frogs and frogs from the Mexican localities. All these crosses produced abnormal embryos. The embryos of the Vermont × lowland crosses were very abnormal and almost completely inviable. The embryos of the Vermont × highland crosses were less abnormal and were completely viable or had a low mortality. The Vermont × Rio Tula embryos were intermediate to the other hybrids in regard to their abnormalities and viability.

4. The adult morphology of the highland, intermediate, and lowland frogs was compared. The highland and lowland frogs are very different from each other. The frogs from the intermediate altitude (Rio Tula) are intermediate in morphology to the frogs from the two extreme altitudes.

5. No difference in size was found between the eggs from El Chico, Zempoala, and San Diego frogs.

6. It is concluded that there is selection for a rapid rate of development at high temperatures in the lowland populations, and that there is no selection for rapid developmental rate in the highland populations.

7. Since there is an environmental gradient from the highlands to the lowlands, it is suggested that the populations of *Rana pipiens* adapt to local conditions along this gradient. Gene flow is also occurring between the populations. Consequently the physiological and morphological characters of the Rio Tula popula-

tion may be the result of gene flow from the high altitude and low altitude populations, as well as from intermediate conditions of selection.

8. It is concluded that further studies of populations from different intermediate altitudes will demonstrate a series of intergrading forms between the highland and lowland populations.

9. It is suggested that the highland Mexican populations have evolved from adjacent lowland populations.

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AN EVOLUTIONARY STUDY OF ISLAND FAUNAS IN THE GULF OF CALIFORNIA, MEXICO, WITH A METHOD FOR COMPARATIVE ANALYSIS

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The potentiality for rapid divergent evolution in small populations has been much discussed in the several recent and well-known works treating evolutionary mechanisms, viz., by Th. Dobzhansky, E. Mayr, J. Huxley, G. G. Simpson, G. L. Stebbins, and others. Mayr (1942: 234–237) has synthesized data for both the invertebrates and vertebrates in which correlations have been established between the size of isolated islands and the variation and differentiation of the island populations. More divergent and usually more homogeneous populations are known to occur on certain smaller islands, and elsewhere where the size of the effective breeding population is relatively small. A theoretical basis for this has been shown by Wright (1931, 1948 and elsewhere), and others, in the relationship of random and directed gene changes to the size of the effective breeding population. "In too small a population there is nearly complete fixation, little variation, little effect of selection and thus a static condition, modified occasionally by chance fixation of rare mutations, leading inevitably to degeneration and extinction" (Wright, 1931).

The herpetofaunae and mammalian faunae of Isla Tiburón and its smaller satellite islands in the Gulf of California has become sufficiently well known to permit a preliminary evolutionary analysis and to warrant certain speculations. In figure 1 (map) the spatial relations of Isla Tiburón, Isla de San Estéban, and Isla Turners[1] are shown. The present analysis is restricted to that of the reptilian and mammalian land species occurring on these three islands; additional comments are made on the lesser known avifauna of this area.

The numbers of endemic species and subspecies of the reptiles and mammals of these islands as presently known are given in table 1. For the mouse *Peromyscus eremicus tiburonensis* Mearns, the opinion of Osgood (1909) rather than that of Burt (1938) has been followed, and this population has not been recognized herein as a subspecies distinct from mainland populations of Sonora. For the remainder of the mammals treated the systematic conclusions of Burt (*op. cit.*) have been followed with full realization that one or more of these species may in reality be especially well marked sub-

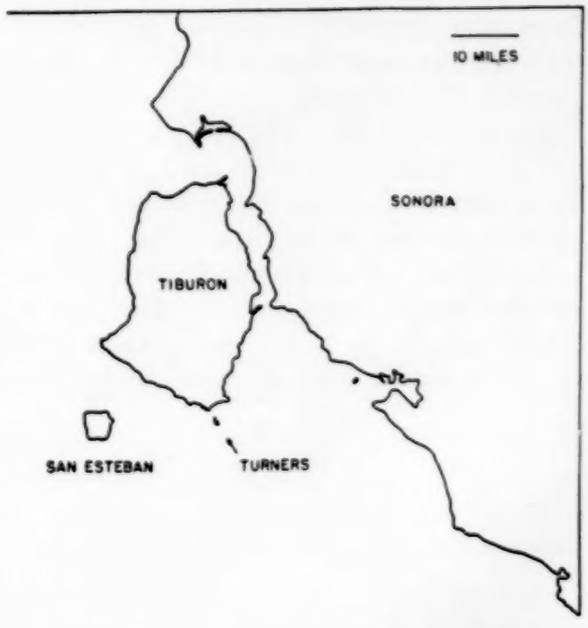

FIG. 1. Geographic relationships of Isla Tiburón, Isla de San Estéban and Isla Turners in the Gulf of California, and the adjacent mainland of Sonora, Mexico.

[1] Tamayo (1949: 579) gives "Isla Turners," and is followed here.

TABLE 1. *Numbers of endemic species and subspecies of reptiles and mammals on three islands in the Gulf of California*

Island	Total number of forms occurring		Endemic subspecies		Endemic species	
	Reptiles	Mammals	Reptiles	Mammals	Reptiles	Mammals
Tiburón	18	9	0	7	0	0
San Estéban	7	1	6	0	1	1
Turners	1	3	1	1	0	2

species; the important point, however, is that these particular populations are allopatric differentiates of respectable genetic divergence. Moreover, such an error of commission does not necessarily constitute an important one in the present system (see table 3, and text below). Recent collecting and study has necessitated a few changes in the systematic status of reptilian populations on Isla San Estéban (Lowe and Norris, 1955) and the description of a dwarfed population of the rattlesnake *Crotalus atrox* found to occur on Isla Turners.

In table 2 the three islands are listed in order of decreasing size. The calculations for mean percent of differentiation as given in the table are based on the scale as presented in table 3. In practice, each entity is assigned one of the four values from 0–100%; the mean value that is calculated therefrom is the mean percent of differentiation for the island fauna considered. The author is fully aware of the limitations of such a schedule. However, its general validity with range of evolutionary divergence from zero to unity is not vitiated and it is supported by the results of practice. One of its advantages is its simplicity. Another is that it does not treat all subspecies in the same manner. Another advantage is the fact that in the problem of species vs. subspecies, should an error be committed in either direction, the difference is 33%; this error, for example, for an island fauna of 10 species and a differentiation percent of 46.6%, affects the calculation by only 3.3% (= 43.3%), a difference that is not significant. Often such errors of interpretation of species vs. subspecies cancel themselves out. Moreover, today and in the future there should be fewer commissions of error in such interpretation as we continue to move away, however slowly it may sometimes seem, from alpha taxonomy.

In figure 2 the numbers of reptile and mammal land species are plotted on the independent variate of island size which is given in square miles, using double logarithmic coordinates. It is observed that the number of species occurring varies in direct relation to the size of the island, with greater richness of fauna on the larger island. This relationship, for these islands, is described by the following equation:

$$N = 4.6A^{+0.268},$$

which is derived from

$$\log N = \log a + b \log A,$$

TABLE 2. *Area, total numbers, and mean per cent of differentiation of land reptiles and mammals on three islands in the Gulf of California*

Figures in parentheses are for reptiles alone and mammals alone, respectively. See tables 1 and 3.

Island	Island area (sq. miles)	Reptiles and Mammals	
		N	% differentiation
Tiburón	466	27 (18+9)	9.8 (0, 29.4)
San Estéban	17.4	8 (7+1)	58.4 (52.4, 100)
Turners	0.4	4 (1+3)	83.5 (67, 89)

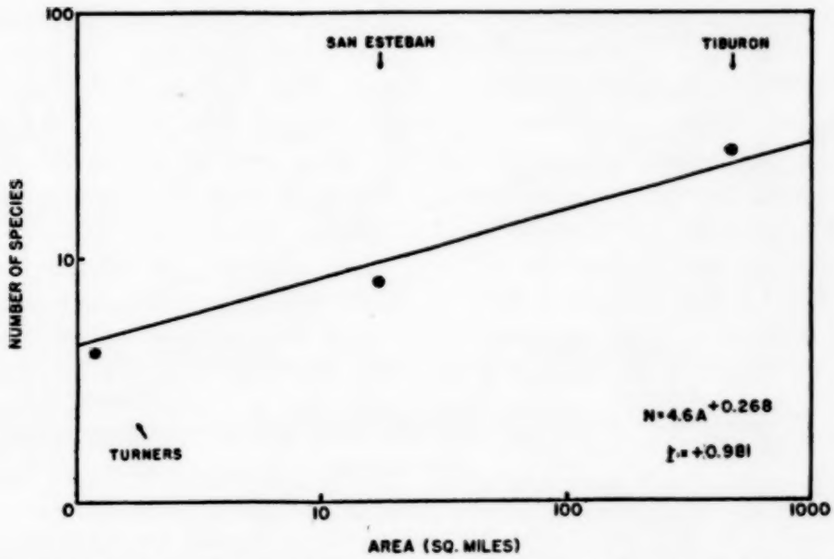

FIG. 2. Regression on double logarithmic scale of numbers of species (of land reptiles and mammals) on total island area, for each of three adjacent islands in the Gulf of California. Data in table 2.

where N is the number of species, A is area in square miles, and a and b the intercept and slope, respectively. The significant (5% level) coefficient of correlation (r) for this regression is + 0.981. The regression lines in figure 2 and 3 were fitted by the method of least squares.

The relationship between the size of the island and the per cent of differentiation of the populations is inverse, as shown in figure 3. The regression of per cent on area is negative and curvilinear as shown on double logarithmic coordinates. The correlation coefficient (r) is − 0.918, and is significant (5% level) for this inverse relationship between degree of evolutionary divergence and population size. The relationship is described by the following equation:

$$\% = 81.1A^{-0.208}.$$

No endemic species (0%) of reptile or mammal is developed on Isla Tiburón as compared to 25% for Isla San Estéban and 50% for Isla Turners. Yet with one exception the genera of all of these endemic species of the smaller two islands occur on the larger Isla Tiburón, the largest island in the Gulf of California.

No known endemic subspecies (0%) of reptile has developed on Tiburón as compared to total divergences of 86% (or 100%) [2] for San Estéban and 100% for Turners.

While the herpetofauna and mammalian fauna of each of these islands is quite well known, there is no reason to believe that the entire numbers of these forms is actually known. With additional field

[2] The systematic status of the population of the lizard *Phyllodactylus tuberculosus* on Isla San Estéban remains in question. It is known from one specimen.

TABLE 3. *Scale of percentages used for calculation of a mean per cent of differentiation for island faunas, as given in table 2 for three islands in the Gulf of California*

Comparisons are made with the related faunas on the adjacent mainland of Sonora, Mexico.

% diff.	Island population character
0	Not recognized as different from (taxonomically same as) mainland or peninsular populations.
33	Endemic subspecies differing significantly *only* in color, pattern, or both; not differing significantly in size, proportions, scales, etc.
67	Endemic subspecies differing significantly in character(s) other than, or in addition to, color and/or pattern.
100	Endemic species; not occurring on mainland or peninsula.

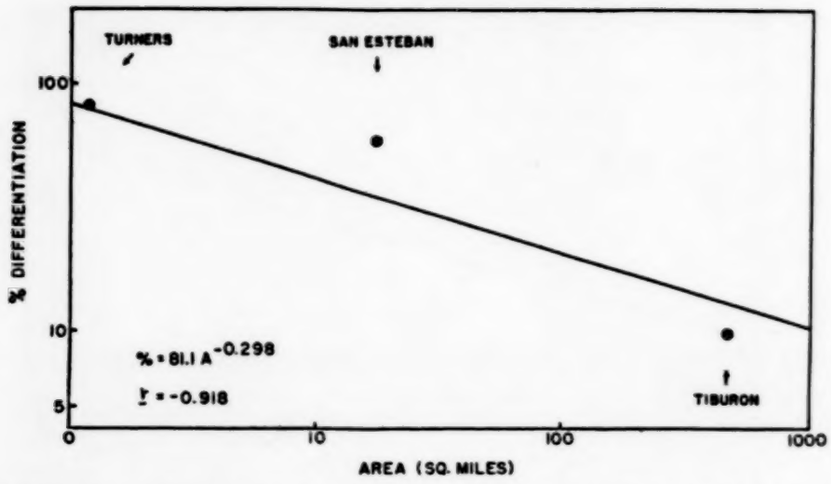

FIG. 3. Regression on double logarithmic scale of per cent of differentiation (of land reptiles and mammals) on total island area, for each of three adjacent islands in the Gulf of California. Data in table 2.

work, it is probable that one or more native reptiles and mammals will be found living on each of these islands. From this analysis it is clear that the probability for such additional forms from Tiburón is that they will be little differentiated, being taxonomically equivalent to mainland populations, or subspecies at most. Those from San Estéban will probably be distinctive, probably subspecifically differentiated, and possibly species. Those from Turners will be distinctive, and probably species if not subspecies.

The land birds of these three islands are less well known, except possibly for Isla Tiburón. Even for the avifauna of Tiburón there remains a question as to the breeding status of several probable residents. Following the works of van Rossem (1932, 1942, 1945), Vaurie (1953), Phillips and Amadon (1952), and discussion with Dr. Alan R. Phillips, there is a total number of 33 resident land birds that can be reasonably assigned as breeding species on Tiburón. Of these 33, six are currently recognized as valid subspecies, differing in color pattern, and there are none that are endemic species. Applying the schedule in table 3, this yields an avifaunal differentiation per cent of 6.0% for resident land birds. Combining these data for reptiles, mammals, and birds presently known to occur on Isla Tiburón, the resulting differentiation per cent is 7.7% which agrees well with the 9.8% for the reptiles and mammals.

The geologic time at which the three islands were formed is not known. Today Isla Tiburón is separated from the mainland by a narrow strait and a water gap at one point of only approximately one mile extent. Isla Turners, the smallest of the three, quite certainly has been fractured off from the closely adjacent Tiburón. Turners lies only approximately one and one-fourth miles off the southwestern corner of Tiburón, and clearly resembles southern Tiburón in general appearance, rock structure, soil type and vegetation type. Turners has probably not been an island for any longer than has Tiburón if, indeed, for as long.

While Isla San Estéban may have been formed prior to both Tiburón and Turners, there is no geological or other evidence to demonstrate this. Its herpetofauna is distinctly Sonoran and not peninsular.[3] Only one native land mam-

[3] This refers to the peninsula of Baja California, Mexico, which lies to the east of Isla San Estéban by approximately the same distance that the Sonoran mainland lies to the west. The Gulf of California at this point is approximately 80 miles wide, as measured E-W along the latitude of the center of the island; it is approximately 55 miles wide as measured in a NE-SW direction through the center of the island.

mal, the endemic species *Peromyscus stephani* Townsend, occurs on the island. Rats (*Rattus*) have reached here and are established. The avifauna is not well enough known to arrive at any satisfactory conclusion as to its affinities, although van Rossem (1945) tentatively considered it more closely peninsular than Sonoran. The known geomorphology and tectonic history of the region (see Beals, 1948) is not sufficient to indicate the historic geological relationship of San Estéban and Tiburón, if there was a close one, and I am of the opinion that there was one. The herpetofauna of San Estéban is similar to and more closely related to that of Tiburón than to that of any other island in the Gulf of California, and is, in fact, *unlike* that of any other than Tiburón. Today these two islands are separated by a relatively short water gap of approximately seven miles.

With regard to the little known geologic history of these islands, it is important for the present study to note that Isla Turners, with the smallest area and greatest degree of faunal differentiation, is essentially a small isolated piece of the largest Isla Tiburón and as such may have been an island for a shorter length of time than Tiburón itself. Moreover, it has probably been isolated for a considerably shorter period of time than has Isla San Estéban.

Summary and Conclusions

A comparative analysis has been made of the apparent evolutionary status of the species of land reptiles and land mammals of three adjacent and differently sized islands in the Gulf of California, Sonora, Mexico; these are, listed in order of decreasing size, Isla Tiburón, Isla de San Estéban, and Isla Turners. A brief comparative analysis of the resident land birds of Isla Tiburón is also presented.

A scale of evolutionary divergence founded on systematic heirarchy and current practice has been constructed and used as a basis for comparative analysis.

It is concluded that the present data demonstrate (1) a direct relationship and significant correlation between island size and numbers of reptile and mammal species occurring thereupon, and (2) an inverse relationship and significant correlation between island size and per cent of differentiation of the animal populations.

The first relationship, of fewer numbers of forms on smaller islands, is inferred to be a correlation between species biomass and area, resulting from restriction of ecological opportunity and the concomitant induced community reorganization upon the complete isolation of the relatively small areas.

The second relationship, of greater genetic differentiation of forms on smaller islands, is inferred to be a correlation between degree of genetic divergence and population size, with more rapid evolution on the smaller islands resulting importantly from increased effect of independent and random gene fixation and loss in spatially isolated small populations without gene exchange with other populations.

The data are compatible with the Neo-Darwinian concept of species formation by accumulation of small increments of genetic change with time, and are, in part, expressive of species formation by extrinsic or geographic (= ecologic) isolation.

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NOTES AND COMMENTS

SOUTH AMERICAN LAND MAMMALS AND THEIR LICE

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The present note is an extract of a longer contribution ("Lice and the history of South American land mammals," *Revista Brasileira de Entomologia*, **3** published in a rather young scientific periodical, which might fail to attract the attention of the general public interested in evolutionary matters. The original paper (also in English) contains sections on the distribution and ecology of South American mammals, systematics of lice and descriptive patterns of infestation, which the authors deem indispensable but are too bulky for inclusion in EVOLUTION.

South American land mammals may be divided into three main groups with respect to the length of their existence in the continent:

1. Mammals which already were in South America when the Panama bridge was interrupted at the Mesozoic-Tertiary transition (Simpson's "ancient immigrants"). This old fauna developed in isolation during most of the Tertiary, radiating into many types of which the modern survivors are the marsupials of the families Didelphidae and Caenolestidae and the edentates (anteaters, armadillos and tree-sloths).

2. Mammals which invaded South America from the north when the Panama bridge was re-established in the Pliocene (Simpson's "late immigrants"). This heavy wave of immigration was followed by widespread extinction of the old fauna, but many of the invaders themselves became rapidly extinct (e.g., elephants, horses, saber-tooth tigers, etc.). Modern representatives are shrews, hares, squirrels, field-mice (Cricetinae), wild dogs, bears, weasels, raccoons, cats, llamas, tapirs, peccaires and deer.

3. Mammals which appear in South America during the Oligocene, without any obvious antecedents in older strata of either North or South America (Simpson's "old island hoppers"). These are the ceboid monkeys and the hystricomorph rodents (porcupines, pacas, pacaranas, capybaras, cavies, agutis, chinchillas, rat-chinchillas and coypus).

The origins of mammals of group 2, the late immigrants, are clear. As to the older South American types, the marsupials seem to be related to North American Late Mesozoic types. The edentates might be descended from North American Paleocene Palaeanodonts, but the evidence is not conclusive. Also probable, but not conclusively proved, is the relationship of the ceboid monkeys to North American lemuroids. The more interesting and debatable case is that of the hystricomorphs. They have been long believed to be related to Old World forms, but Wood has recently opposed this viewpoint, although not conclusively.

We attempt to determine whether the patterns of infestation with lice (Mallophaga and Anoplura, biting and sucking lice) might not afford clues to the origin and relationships of these mammalian groups, as well as other evolutionary data both on hosts and parasites.

To establish the patterns of infestation we started by compiling a list of the lice definitely known to parasitize South American land mammals. Then, we compiled a list of the closest relatives of the parasites of each mammalian group, separately. It is essential to note that such relationships were evaluated solely on the basis of morphological characters; no data from host-relationships were used. The next step was to list the mammals parasitized by the aforesaid closest relatives, and the final one to check the relationships, systematical and geographical, of the latter mammals with the original host-group. As an example:

South American marsupials are parasitized solely by lice of the genus *Cummingsia* (Mallophaga, Trimenoponidae). Two of the three known species of *Cummingsia* parasitize didelphids. The third lives on a caenolestid. It is well established that didelphids and caenolestids were already well differentiated in the Paleocene, and even then their taxonomic relationships were not close. Otherwise, the geographical distribution of the species of *Cummingsia* is widely disjunct. One species is restricted to the Andes of Peru, another to one small area in eastern Brasil, and the third one to a wider area in northeastern Brasil. Other trimenoponids occur on South American hystricomorphs, one species parasitizing secondarily one hare. No trimenoponids are known outside of South America. On the family level, the closest relatives of the Trimenoponidae are the Boopidae, parasites of Australian marsupials.

Let us see what can be inferred from the facts listed above. First, we see that the parasites of South American marsupials belong to a strictly South American family, and that they occur also on hystricomorphs. The geographical distribution of the trimenoponids points to

their origin on the marsupials and to subsequent contamination of the rodents. Then we see that the trimenoponids are related, on the family level, to parasites of Australian marsupials. The final conclusion is that the infestation of South American marsupials is a primary one and also very ancient, as both didelphids and caenolestids, geographically disjunct, are infested. In support of this hypothesis, we may say that the trimenoponids are well differentiated from other marsupiophile lice, although still showing very clearly their relationships.

Consistent application of this type of reasoning led to the following conclusions:

1. It is possible to correlate patterns of infestation and origin of South American land mammals.

2. The late immigrant mammals are characterized by the presence of parasites closely related to those of their relatives in other parts of the world (especially North America). In some cases one single species (e.g. some lice of squirrels) or genus (e.g. lice of otters and camels) occur in wide areas, encompassing more than one continent. In some cases late immigrant mammals have acquired lice from the older South American fauna, but such cases are very easy to detect. The more striking cases are the acquisition by peccaries of hystricophile lice, and by hares of caviiphile lice.

The pattern characterized by the presence of world-wide Anoplura and trichodectid Mallophaga as primary infestants, and by the absence of gyropid and trimenoponid Mallophaga, also as primary infestants, we have called, by analogy, "late immigrant pattern."

3. South American marsupials are parasitized (as described above) by strictly South American lice, related on the family level to lice of Australian marsupials. This brings no new data on the origin of South American marsupials (as there is nothing to show whether the relationships between South American and Australian marsupials are direct or *via* extinct northern forms), but serves well to show the reliability of the method used, since strict morphological and distributional criteria lead to a very plausible conclusion, well in agreement with the bulk of existing evidence.

4. The tree-sloths (Bradypodidae) are the only edentates known to be parasitzed by lice (*Lymeon,* Mallophaga, Trichodectidae). The trichodectids are a widespread and diversified group, seemingly very ancient, which parasitize an enormous variety of animals over the entire world. The parasites of the sloths are not obviously related to any known group of trichodectids, so there is nothing to be said about relationships of the group. Nevertheless, the presence of an isolated stock of trichodectids is an important fact.

5. The ceboid monkeys are parasitized by Anoplura and Mallophaga. Their sucking lice belong to the genus *Pediculus,* the taxonomy of which is so confused that we do not wish to use it in the present connection. It seems possible that monkey *Pediculus* were acquired from pre-Columbian man. The biting lice of the ceboids belong to the genus *Cebidicola* (Trichodectidae), which is said, on strictly morphological grounds, to be related to *Lorisicola,* of Oriental lorises. Here we see again the presence of a differentiated trichodectid stock, not so isolated as *Lymeon,* but pointing to a plausible relationship. The lice of Old World monkeys show no direct relationship to those of the ceboids.

6. The situation of the hystricomorphs is more complex, as they are widely and intensively parasitized. The following general facts should be stressed:

a) The porcupines (Erethizontidae) are parasitized by an isolated stock of trichodectids (*Eutrichophilus*), a situation resembling that which obtains for the sloths.

b) The primary infestants of the remainder of the hystricomorphs are gyropid and trimenoponid Mallophaga. The latter have already been discussed with the marsupials: a strictly South American group, originally marsupiophile. The gyropids, however, seem to have developed on the hystricomorphs, from which they have contaminated other groups, especially cricetines and lagomorphs, with moderate success. They are also a strictly South American group.

c) Sucking lice are found on hystricomorphs, but it is easy to see that they are secondary infestants, acquired from late immigrant cricetines. We cannot present here a discussion of the numerous forms involved, but do not doubt that they are late immigrants. This fact has some important implications, to be discussed below.

d) The lice of Old World hystricomorphs are not directly related to those of South American forms. This is an indirect confirmation of Wood's hypothesis of independence of the two groups. The consistent agreement between patterns of infestation and accepted phylogenetic relationships in all other groups investigated adds strength to this negative evidence.

7. It is not possible at present to define separate patterns of infestation for "ancient immigrants" and "old island hoppers." We are forced to lump them in one single category, which we choose to call "autochton," since Dunn's prior term "Old Southern" is not translatable in our native language. The autochton pattern is characterized by the presence, as primary infestants, of trimenoponid and/or gyropid Mallophaga, of specialized trichodectid Mal-

lophaga and by the absence, also as primary infestants, of Anoplura.

8. The absence of Anoplura as primary infestants of South American land mammals seems to show that the sucking lice, as a group, originated at the earliest during the very late Mesozoic, if not at the beginning of the Tertiary. The reasons for this inference are:

a) If sucking lice existed before the Pliocene in South America, they must have suffered complete extinction. This is hard to believe, especially because autochton rodents have proved very suitable hosts for cricetine-born Anoplura. As a matter of fact, the exchange of parasites has been much more intense in the cricetine-hystricomorph direction than otherwise.

b) If sucking lice existed in North America during the Paleocene, it is hard to believe they did not follow the heavy wave of immigration which entered South America at the time.

c) Absence of sucking lice in North America at the Mesozoic-Tertiary boundary could only be explained by either non-existence of the group or a very restricted distribution. This because the early Tertiary was a time of intensive faunal movements in Holarctica.

d) If the Anoplura at that time had a restricted distribution, this should be due to very young age of the group, as they have proved very aggressive and succesful in colonizing empty ecological niches.

9. The possibility of detecting secondary infestations implies the possibility of evaluating evolutionary rates, as we are in a position to assign maximum lengths to the processes of divergence. Further knowledge of faunal movements inside South America will enhance the precision of such estimates.

10. We believe it would be rewarding to apply the methods outlined above to the fauna of other areas, especially the Oriental region and Africa. Otherwise, similar methods might be used for other couples of host-parasite groups, provided adequate taxonomic knowledge is available.

Acknowledgments. The authors are indebted to a great many friends for help in preparing the paper of which this is an extract; these friends are listed in the article in Revista Brasileira de Entomologia. At the present time the authors wish to state their gratitude to the Editor of EVOLUTION for this opportunity of presenting their data to a much wider public than it would be possible otherwise.

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SELF-COMPATIBILITY AND ESTABLISHMENT AFTER "'LONG-DISTANCE" DISPERSAL

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It is not often that research on an evolutionary topic carried out independently by botanists and zoologists produces conclusions which are virtually identical. When this does happen one cannot restrain a feeling that a principle of more than superficial importance has been uncovered. It is one of the functions of this journal and of the Society which publishes it to make it possible for workers in separate fields to see the common significance of their results and, for one botanist at least, this object has been achieved with the publication of a recent paper by zoologist A. R. Longhurst (1955).

Because immobility and hermaphroditism pre-

vail in the Plant Kingdom, comparative considerations of the reproductive biology of plants and animals are most appropriately made when the zoological representatives are more or less sedentary invertebrates, particularly those in which sexual dimorphism is not firmly fixed. It is for this reason that Dr. Longhurst's examination of the Notostraca has a particular importance for botanists. His studies have led him to some conclusions which are in the closest accord with those which I first published seven years ago (Baker, 1948) after examining members of the angiosperm family Plumbaginaceae. These latter conclusions, based exclusively on flowering-plant material, have subsequently been illustrated and used as a basis for argument in a number of papers (particularly Baker, 1953*a*, 1953*b*).

The Notostraca are themselves confined to temporary pools of water but their eggs may be passively distributed over large distances by wind or attached to higher animals. This is quite comparable with the dispersal of higher plants by the distribution of their seeds. Therefore it is of the greatest interest that, whereas in flowering plants it has been possible to demonstrate some correlation between occurrence in localities most likely to have been reached by more or less "long-distance" dispersal and the development of self-compatibility (even in groups where self-incompatibility prevails), in the normally dioecious Notostraca there is a comparable correlation with derived hermaphroditism.

We have both put forward the same explanation of the correlation. With self-compatible individuals a single propagule is sufficient to start a sexually-reproducing colony, making its establishment much more likely than if the chance growth of two self-incompatible yet cross-compatible individuals sufficiently close together spatially and temporally is required. In addition, self-compatible flowering plants are usually able to form some seed in the absence of visits from specialized pollinating insects, which may be absent from the new situation. For animals autogamous hemaphroditism provides the mechanism for ready establishment; with plants apomixis and purely vegetative reproduction are also available to replace self-incompatible hermaphroditism (and monoecism) or dioecism.

A comprehensive review of this subject as it concerns plants is in preparation (although at a slower rate than could be desired by reason of my present location away from major libraries). In the meanwhile, however, it may be appropriate to remark that although there are many exceptions (for one thing not all disjunct distributions are the result of long-distance dispersal), supporting evidence for the thesis outlined above may be found in at least a score of families of flowering plants. Taylor (1954) has recently described a clear case in *Coprosma* (Rubiaceae) where, on Macquarie Island, in the Antarctic Ocean, *C. pumila* Hook. it exists in a monoecious form contrasting with its characteristic dioecious condition in New Zealand.

Some of the best evidence comes from a study of the reproductive biology of weeds (which are pre-eminently plants that are able to effect opportunist range-expansions of a discontinuous nature). They are also able to survive periodic decimations of their populations (which demands the ability to regenerate a population from very few survivors or even from a single individual). It is probably not a coincidence that the vast majority of weeds are self-compatible or possess strong powers of vegetative reproduction.

In the Pteridophyta, it seems likely that Raunkiaer's (1920) observation that the floras of oceanic islands contain remarkably high proportions of Pteridophyta may be explicable not only in terms of favourable climatic conditions or the possession by these plants of light wind-dispersed spores, as he suggests, but also of the hermaphrodite nature of the fern gametophyte. Only one case of possible self-incompatibility in ferns is known (Wilkie, in Bateman, 1952), and it is the usual experience of those who have to hybridize these plants that self-fertilization is very difficult to avoid. In the Brophyta there is a similar suggestion of contrast between the world-distribution patterns of dioecious and other taxa, but there are many complicating factors.

The cooperation of botanists in suggesting groups of taxa which might be investigated from this point of view is invited. Similarly, it is suggested, in all humility, that other zoologists might follow the example of Dr. Longhurst and examine the distribution-patterns of selected invertebrate groups in relation to their sexuality.

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TENTH ANNUAL MEETING: SOCIETY FOR THE STUDY OF EVOLUTION

UNIVERSITY OF TEXAS, AUSTIN, TEXAS

April 8–11, 1955

The success of the Tenth Annual Meeting will be attested by all who were fortunate enough to attend. The program was full but not crowded and ample time was available for discussion. Total attendance was about 125 (101 registered: 62 from Texas; 39 from other states). The all day field excursion on Saturday to the Gulf Coastal Plain and the Edwards Plateau was a successful occasion, despite a lack of full cooperation from the weather, for it not only illustrated the wealth of ecological diversity in the environs of Austin, but also provided a stimulating setting for innumerable informal discussions. The dinner and barbeque supper were both gastronomic successes. The Local Committee, under the chairmanship of W. Frank Blair, is certainly to be congratulated on arranging such an outstanding meeting.

BUSINESS MEETING, APRIL 10

The meeting was called to order by President Sewall Wright.

The Secretary reported on the action taken by the Council at their earlier meeting including the decision to hold the Twelfth Annual Meeting of the Society at Stanford University, September, 1957, in conjunction with the A.I.B.S. (The Eleventh Annual Meeting is to be held in New York, December, 1956, with the A.A.A.S.). The Council also accepted the invitation of the Genetics Society of America to cosponsor the Tenth International Congress of Genetics, to be held at McGill University, Montreal, Canada, August 20–27, 1958.

The Treasurer reported on the healthy financial condition of the Society. Subsequently a motion was passed to the effect that the Council be urged to increase the budget of EVOLUTION by the amount received as interest on investments.

The Editor of EVOLUTION presented an informal report on the status of the journal. The number of acceptable papers has been increasing, with a consequent increase in the length of time between receipt of manuscripts and publication. Of particular interest is the increase in papers originating outside of the United States from 16 to 40 per cent in recent years (based upon senior author). A discussion was initiated concerning the desirability of accepting papers in languages other than English. No action was taken, although the Editor indicated that he would solicit an opinion on this matter from the Associate Editors and report to the Society at a later date.

J. T. Patterson was elected to fill the vacancy on the Council created by the death of E. B. Babcock.

PAPERS, LECTURES, AND DEMONSTRATIONS PRESENTED AT THE AUSTIN MEETING

Alexander, Mary L., University of Texas. Radiation effects in immature germ cells.

Bennett, Jack, University of Oklahoma. A wing venation variant in wild *Drosophila tripunctata* Loew.

Bradshaw, Newman, University of Texas. Species discrimination in the *Peromyscus leucopus* group of mice.

Bruneau, L. Herbert, University of Texas. Population cage analyses.

Buri, Peter, University of Chicago. Effective population size in small populations of mutant *Drosophila*.

Christensen, Eleanor, R. D. Owen, and J. B. Loefer, California Institute of Technology. Some aspects of the serology of *Tetrahymena*.

Crow, James F., University of Wisconsin. Analysis of a DDT-resistant strain of *Drosophila*.

DeBusk, A. Gib, University of Texas. Mutations and *Neurospora* populations.

Dowling, Herndon G., University of Arkansas. Evolution in serpents.

Epling, Carl, University of California, Los Angeles. A critique of adaptive polymorphism, balanced euheterosis, and coadaptation.

Grant, Verne, Rancho Santa Ana Botanic Garden. The genetic basis of racial and specific differentiation in *Gilia* (Polemoniaceae).

Haller, John R., University of California, Los Angeles. The relationship of *Pinus ponderosa* and *Pinus jeffreyi*.

Heed, William B., University of Texas. Dis-

tribution and populations of the *Drosophila* of Salvador.

Hubbs, Carl, Scripps Institution of Oceanography. Genetic differentiation as indicated by breeding experiments and natural hybridization in fishes.

Hubbs, Clark. University of Texas. Hybridization between Texas freshwater fishes, and small population variation in Gambusia.

Irwin, M. R., University of Wisconsin. Interrelationships and evolutionary patterns of cellular antigens in Columbidae.

Judd, Burke, University of Texas. Position effect studies with *Drosophila.*

Kennerly, T. E., University of Texas. Contact between ranges of two allopatric species of pocket gophers.

Krivshenko, Jakov, University of Rochester. Cytogenetic study of the X-chromosome of *Drosophila busckii,* and its relation to phylogeny.

Lewis, Harlan, University of California, Los Angeles. The role of gross chromosomal alterations in speciation in *Clarkia.*

Lewis, Harlan, University of California, Los Angeles. Phylogeny of the plant genus *Clarkia.*

Levine, R. P., and Elizabeth E. Levine, Harvard University. Gene recombination in populations of *Drosophila pseudoobscura.*

Levine, R. P., and Elizabeth E. Levine, Harvard University. The effect of low humidity on the oocytes of *Drosophila.*

Lower, Wm. R., University of California, Los Angeles. Changes within an inversion system of *Drosophila pseudoobscura* during 100 generations.

Mattoni, R. H. T., University of California, Los Angeles. The relation of individual viability to range of tolerance and its importance in stabilizing selection.

Meacham, W. R., University of Texas. Laboratory hybridization of *Bufo woodhousei* and *B. fowleri.*

Mecham, John S., University of Texas. Dispersal in *Pseudacris streckeri* and its relation to local distribution of an allelomorphic color variant, and hybridization in hylid frogs.

Mettler, Lawrence E., University of Texas. Population cage analyses, and movements of Drosophila in wild populations. Demonstration of Kodachromes showing colors in horses.

Mickey, George H., Northwestern University. Some relationships of heterochromatin to evolution.

Miller, Alden H., University of California, Berkeley. Ecologic factors that accelerate formation of races and species of terrestrial vertebrates.

Miller, Dwight D., University of Nebraska. Sexual isolation between eastern and western *Drosophila athabaska.*

Mitchell, Donald F., Pennsylvania State University. Conformity of inversion homozygotes and heterozygotes to the expectations to the Hardy-Weinberg Rule in wild populations of *Drosophila pseudoobscura.*

Mooring, John, University of California, Los Angeles. Chromosomal rearrangements in wild populations of *Clarkia unguiculata.*

Patterson, J. T., University of Texas. Species of *Drosophila.*

Pettus, David, University of Texas. Typostrophic variation in the banded water snake.

Pyburn, W. F., University of Texas. Local differentiation in color pattern of cricket frogs (*Acris*) in relation to population size and distribution pattern.

Ross, Herbert H., Illinois Natural History Survey, Urbana. Evolution of temperature tolerance in the Trichoptera.

Seiger, M. S. B., and R. H. T. Mattoni, University of California, Los Angeles. Restrictions on the flight of individuals by small ecological barriers in a butterfly population.

Shapard, Pauline, University of Texas. Biochemical studies of pseudoallels in *Drosophila.*

Snow, Richard, University of California, Los Angeles. The origin of aneuploidy in wild populations of *Clarkia unguiculata.*

Spencer, Warren P., College of Wooster. Genetic mechanisms in *Drosophila* speciation.

Stalker, Harrison D., Washington University. Parthenogenesis in *Lonchoptera dubia.*

Straw, Richard M., Rancho Santa Ana Botanic Garden. Floral isolation in *Penstemon.*

Strawn, Kirk, University of Texas. Temperature influenced variations of meristic characters of the greenthroat darter (*Etheostoma lepidum*), and hybridization between Texas freshwater fishes.

Tamsitt, J. R., University of Texas. Species discrimination in the *Peromyscus truei* group of mice.

Tinderholt, Victor E., University of California, Los Angeles. A theory of balanced polymorphic populations.

Volpe, E. Peter, Tulane University. A taxogenetic analysis of the status of *Rana kandiyohi* and *Rana burnsi.*

Wagner, Robert P., University of Texas. Demonstration of chemical interaction of genes.

Wasserman, A. O., University of Texas. Laboratory hybridization of spadefoots (Genus *Scaphiopus*).

Wheeler, Marshall R., University of Texas. Exhibition of specimens of North American Drosophilidae.

Wilson, J. A., University of Texas. Evolution in successive Miocene mammalian fuanas of the Texas Gulf Coast.

Wright, Sewall, University of Wisconsin. Modes of selection.

Young, Keith, University of Texas. Invertebrate paleontology laboratory.

PAPERS PRESENTED AT THE EAST LANSING, MICHIGAN MEETING, SEPTEMBER, 1955

Bailey, Joseph R., Duke University. The taxonomic level of adaptive radiation.

Brattstrom, Bayard H., California Institute of Technology. Evolution and zoogeography of the pit-vipers (Reptilia: Crotalidae).

Löve, Áskell, University of Manitoba. Sex determination in *Rumex*.

Madison, K. M., University of Illinois. The possible role of the pyrophosphates in the primitive inorganic organism.

Michener, Charles D., and Robert R. Sokal, University of Kansas. A quantitative approach to a problem in classification.

Moody, Paul A., and David E. Doniger, University of Vermont. Serological light on porcupine relationships.

Sharp, Aaron J., University of Tennessee. Evolution and preparation of local floras.

Spiess, Eliot B., University of Pittsburgh. Adaptive values of certain *Drosophila persimilis* gene arrangements from experimental populations.

HARLAN LEWIS,
Secretary

CORRIGENDA

The title of the article by Max Levitan, Volume IX, number 1, pp. 62–74, was incorrectly given on the title page as follows:

Studies in Linkage in Populations: I. Associations of Second Chromosome Linkages in *Drosophila robusta*.

The correct title, as given in the article, is:

Studies of Linkage in Populations. I. Associations of Second Chromosome Inversions in *Drosophila robusta*.